SYNTHETIC STUDIES ON THE A83586C / GE3 FAMILY OF ANTITUMOUR ANTIBIOTICS

By

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This thesis is dedicated to my family in particular my parents for their continued love and support throughout my life, without which I would never have achieved my goals. I wish to also dedicate my thesis to my loving fiancée whose love and devotion was a major inspiration to the accomplishment of my research work. For this, I am indebted to them forever.

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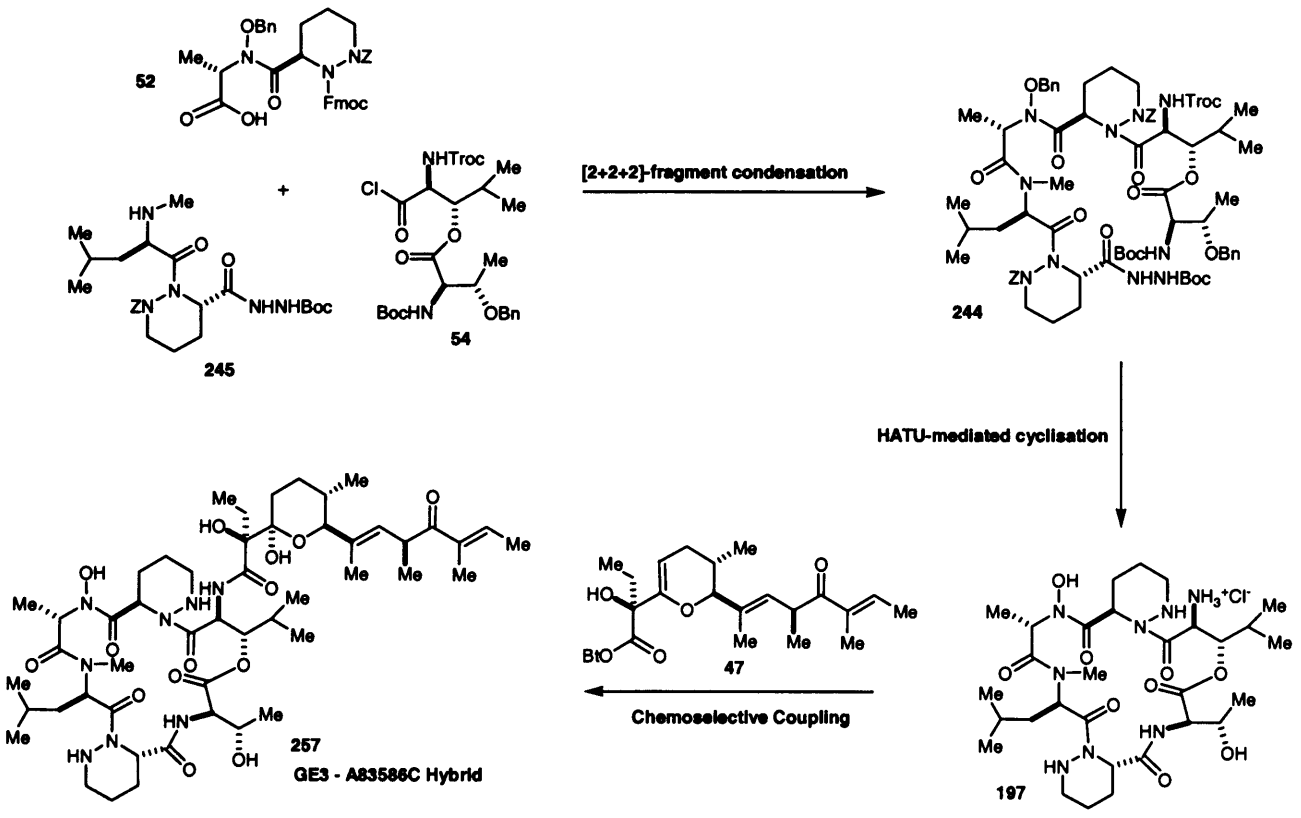
ABSTRACT

The A83586C/GE3 class of cyclodepsipeptides are a family of antitumour antibiotics whose antitumour properties have been attributed to their ability to inhibit E2F transcription factors. The latter are critical regulators of cellular proliferation that are potentially important new therapeutic targets for the control of proliferative diseases such as cancer.

In this thesis, the asymmetric total synthesis of several A83586C/GE3/Verucopeptin analogues is described. Some of these molecules have provided valuable insights into the actual mechanism of antitumour activity for this class. All of these molecules have been built through a chemoselective coupling strategy involving the fully elaborated pyran *N*-hydroxybenzotriazole activated ester **47** and the relevant unprotected cyclohexadepsipeptide. The latter were each synthesised through a [2+2+2]-fragment condensation strategy and macrolactamisation was accomplished with HATU. The approach used is exemplified below by our synthesis of the A83586C/GE3 hybrid **257**.



CONTENTS

ABBREVIATIONS

AcCl	acetyl chloride
AcOH	acetic acid
AIBN	azobisisobutyronitrile
Alloc	allyloxycarbonyl
Boc	*tert*-butyloxycarbonyl
Boc_2O	Di-*tert*-butyl dicarbonate
BOP	(1-benzotriazolyl)-oxy-tris(dimethylamino) phosphonium hexafluorophosphate
BOPCl	bis-(2-oxo-3-oxazolidinyl)phosphinic chloride
Bt	benzotriazole
DBAD	di-*tert*-butyl diazodicarboxylate
DCC	dicyclohexyl carbodiimide
DDQ	2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone
DET	diethyl tartrate
DIBAL	diisobutyl aluminium hydride
DMAP	4-dimethylamino pyridine
DMF	*N*, *N*-dimethylformamide
DMP	Dess-Martin Periodinane
DMPU	1, 3-dimethyl hexahydro-2-pyrimidinone
DMSO	dimethyl sulfoxide
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EtOAc	ethyl acetate
Fmoc	(9H-fluoren-9-ylmethoxy)carbonyl
HATU	*O*-(7-azabenzotriazol-1-yl)-*N*, *N*, *N'*, *N*-tetramethyluronium hexafluorophosphate
HMPA	hexamethyl phosphoramide
HOBt	1-hydroxy 1H-benzotriazole
KHMDS	potassium hexamethyldisilazide
LDA	lithium diisopropylamide
MeCN	acetonitrile

MsCl	methanesulfonyl chloride
NaHMDS	sodium hexamethyldisilazide
NBS	*N*-bromosuccinimide
NEM	*N*-ethylmorpholine
NMO	4-Methylmorpholine *N*-oxide
Oxone	Potassium monopersulfate
PDC	pyridinium dichromate
PMB	4-methoxybenzyl
PMBOH	4-methoxybenzyl alcohol
PPTS	pyridinium *p*-toluene sulfonate
PvCl	pivaloyl chloride
REDAL	sodium bis(2-methoxyethoxy) aluminium hydride
TBAF	tetrabutyl ammonium fluoride
TBDMS	*tert*-butyl dimethyl silyl
TBDPS	*tert*-butyl diphenyl silyl
TBHP	*tert*-butyl hydroperoxide
TBS	*tert*-butyl dimethyl silyl
Teoc	*N*-(trimethyl-silyl) ethoxycarbonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMS	trimethylsilyl
TPAP	tetrapropylammonium perruthenate
Troc	trichloro-ethoxycarbonyl
TsOH	4-methyl benzenesulfonic acid
Z	benzyloxycarbonyl
ZCl	benzyl chloroformate

CHAPTER 1 THE A83586C/GE3 FAMILY OF ANTITUMOUR AGENTS

Cyclodepsipeptides are cyclic peptides with one or more ester bonds. A large number of naturally occurring cyclodepsipeptides have been encountered over the past 20 years. Many are of great scientific interest for a number of reasons. First, many of these molecules are medicinally valuable as a result of their antimicrobial and antitumour effects. Second, many cyclodepsipeptides are useful interrogator molecules that can be used to perturb and investigate the signalling pathways that regulate cell functioning. Finally, many cyclodepsipeptides have synthetically challenging molecular architectures that stimulate new reaction development.

Of special interest to many groups are cyclodepsipeptides of the A83586C/GE3 family (Figure 1). These have been the focus of a considerable amount of research by our group over the past decade. This effort has been driven primarily by their significant antitumour effects, their postulated ability to regulate E2F transcription factors and, in some cases, their capacity to induce apoptosis in apoptosis-resistant cancer cell lines. Members of this family are all structurally characterised by a 19-membered cyclodepsipeptide ring linked to a tetrahydropyranyl side chain. All possess a conserved tripeptide motif in which an *N*-hydroxyamino acid, (3*R*)-piperazic acid, and (2*S*, 3*S*)-3-hydroxyleucine are all linked together. Moreover, a tetrahydropyranyl side chain is always linked to the cyclodepsipeptide ring via the hydroxyleucine nitrogen.

In the coming section, we will discuss the isolation, the structure elucidation, and the biological properties of various family members.

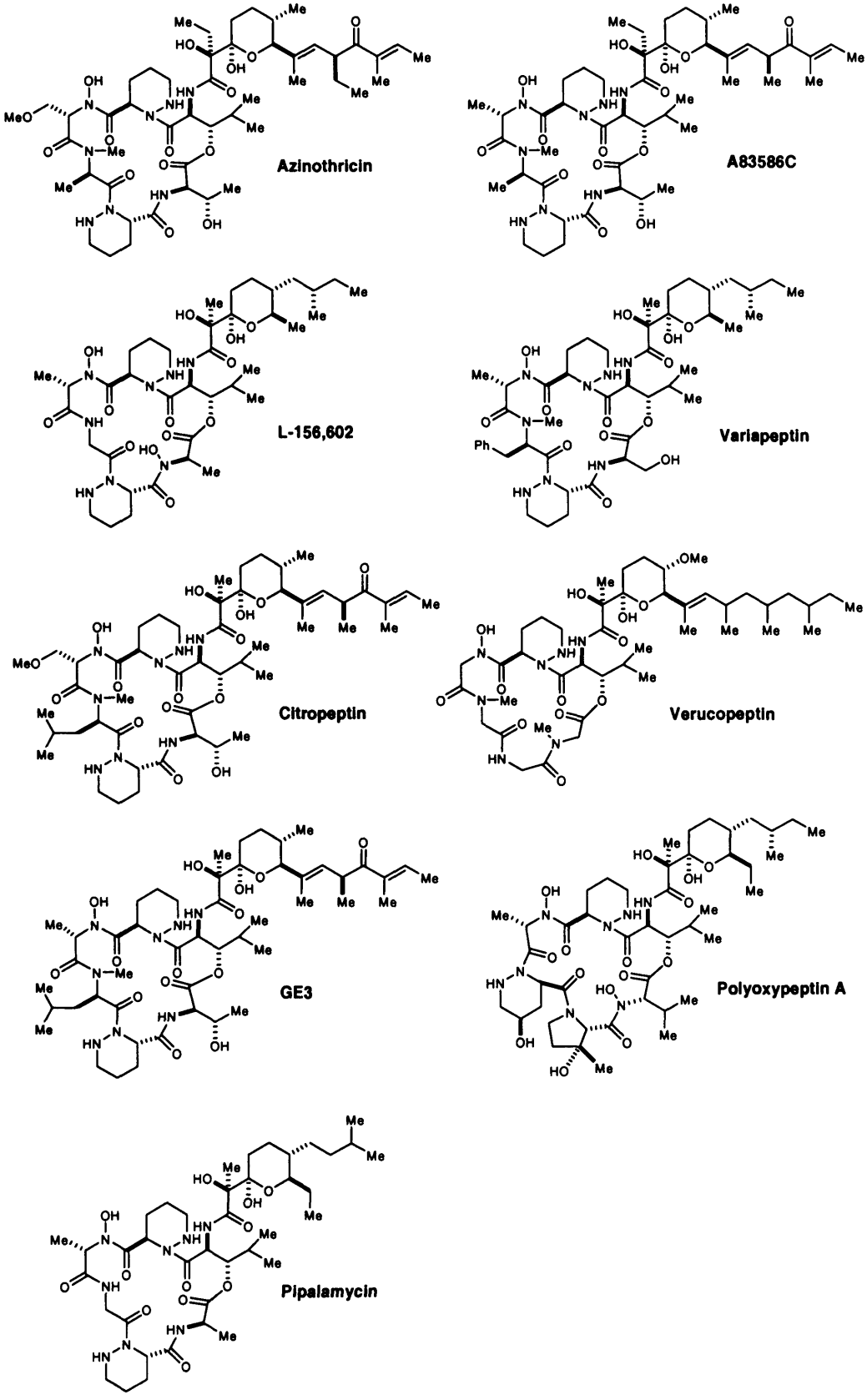
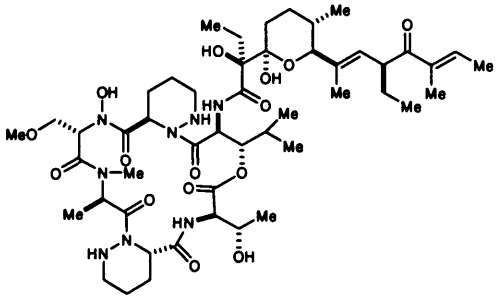


Figure 1 The A83586C/GE3 Family of Pyranylated Cyclodepsipeptides

1.1 Isolation and Biological Activity

In 1986 Maehr and coworkers[1] at Hoffmann La Roche reported their discovery of azinothricin in culture filtrates of *Streptomyces sp. X14950*, during their search for new compounds with powerful antibiotic effects. Azinothricin was the prototype of this class and was one of the most potent antibiotics ever discovered in the Roche natural product screening assay. Its activity profile was primarily directed against resistant Gram-positive strains of bacteria. In terms of potency, its MIC[†] values ranged from <0.008 to 0.016 μg/ml for 31 strains of *Staphylococcus aureus*; 0.063 μg/ml for 16 strains of *Enterococcus faecalis*; 0.016 μg/ml for 2 strains of *Streptococcus pyogenes*; and <0.008 μg/ml for 2 strains of *Streptococcus pneumoniae*. Azinothricin was also active against the two anaerobes *Clostridium histolyticum* and *Clostridium septicum* with MIC values of 0.001 μg/ml, but was much less effective against Gram-negative bacteria and fungi. Toxicity studies in mice (Figure 2) revealed that azinothricin was lethal at high drug concentrations which ultimately prevented its clinical development as an antibacterial drug.



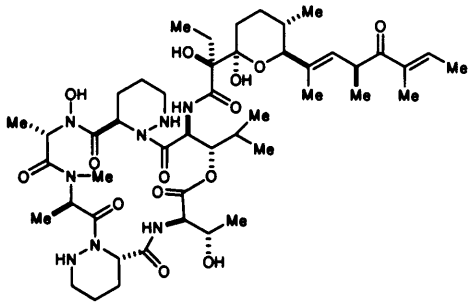
Administration	LD_{50}[‡] mg/kg / in mice
Intravenous	10
Intraperitoneal	3.2
Sub-cutaneous	420
Oral	>500

Figure 2 Toxicity profile of azinothricin

In 1988, the structure of a second member of the class was elucidated by X-ray crystallography and chemical degradation. The new molecule was termed A83586C and it was isolated from the fermentation broths of the Guam soil micro-organism *Streptomyces karnatakensis* by Smitka and colleagues at Eli Lilly.[2] The absolute configuration of A83586C was confirmed by the detection of D-threonine in its acid hydrosylate. A total of 10 mg of the natural product was isolated

[†] Minimum Inhibitory Concentration; the lowest concentration of material that prevents visible bacterial growth
[‡] Lethal Dose; amount of material that it takes to kill 50% of test animals in one dose

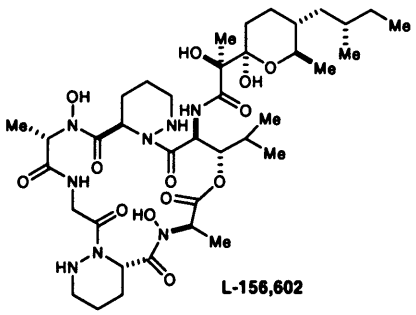
from 10 litres of broth. Again, A83586C showed extremely potent activity *in vitro* against Gram-positive organisms (Figure 3), but was inactive against Gram-negative bacteria. A83586C was also a remarkably effective antitumour agent with an IC_{50}[†] value of 0.0135 μg/ml *in vitro* against a CCRF-CEM human T-cell leukaemia line. Again, toxicity issues at high drug concentrations prevented its clinical development as an antibacterial drug. Thus at doses of 9.3 mg/kg, A83586C was lethal to mice infected with *Staphylococcus aureus.*



Organism	MIC (μg/ml)
Staphylococcus aureus	0.008
Staphylococcus epidermidis	0.008
Streptococcus pyogenes	0.008
Streptococcus pneumoniae	0.008
Streptococcus faecalis	0.06

Figure 3 Antimicrobial activity of A83586C

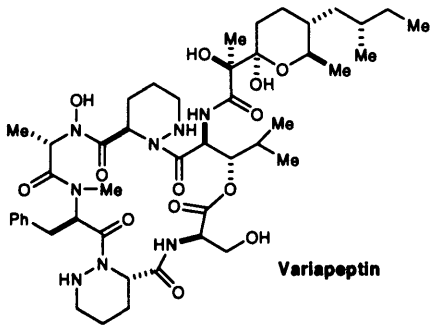
In 1990 three other molecules of this genre were isolated and subsequently characterised. These were: L-156,602, variapeptin and citropeptin. L-156,602 was isolated from culture broths of *Streptomyces spp. MA6348* by Hensens and co-workers[3] at Merck Sharp and Dohme Research (MSD) Laboratories. L-156,602 was found to competitively inhibit the binding of the C5a anaphylatoxin to its receptor on human polymorphonuclear leukocytes. C5a has been implicated as a causative or aggravating agent in a variety of inflammatory and allergic diseases, and hence an inhibitor of this protein would be potentially beneficial for treating such ailments. With synthetic analogue development in mind, Charles Caldwell and Phillipe Durette led an MSD chemistry team that synthesised L-156,602 in 1990 (their synthesis will be discussed later).[69, 70]

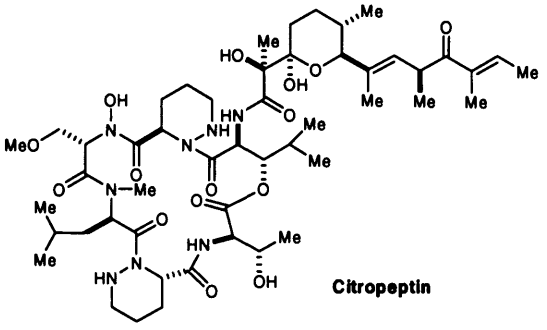


L-156,602

[†] Inhibitory Concentration; concentration of a drug that is required for 50% inhibition of tumour growth in test animal

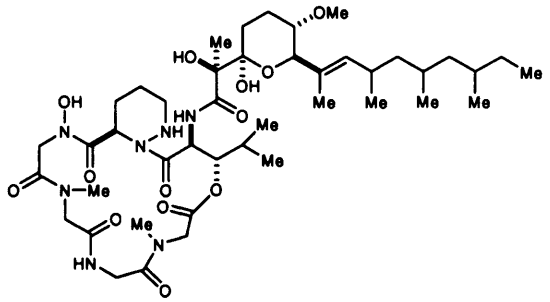
Variapeptin[4,6] and citropeptin[5,6] were isolated from the fermentation broths of *Streptomyces variabilis* and *Streptomyces flavidovirens* respectively and their structures were determined by NMR comparisons with A83586C and azinothricin. Variapeptin showed very potent activity *in vitro* against Gram-positive bacteria but no activity against Gram-negative bacteria, or fungi. More importantly, however, variapeptin exhibited antitumour activity against P388 leukaemia cells (IC_{50}= 0.01 μg/ml) but again was found to be toxic to mice when administered intraperitoneally at 5 mg/kg. Moreover, at the highest non-toxic dose it was inactive *in vivo* against P388 lymphocytic leukaemia. Fortunately the biological activity of citropeptin proved to be more encouraging. It was found to confer a 120% life extension when administered to mice with P388 lymphocytic leukaemia at the non-lethal dose of 2 mg/kg/day. This key discovery indicated, for the first time, that molecules of this class could be administered at non-toxic low dosages, and this immediately revived biological interest in these molecules as potential anticancer agents. This interest was further reinforced by the isolation and biological screening of subsequent family members.





The next member of this family to be discovered was verucopeptin in 1993. It was isolated from the Philipino soil organism *Actinomadura verrucospora Q886-2* by Nishiyama and coworkers[7,8,9] during a search for novel microbial metabolites with potent antitumour activity. The structure of verucopeptin was determined by spectroscopic analysis and chemical degradation studies. Nishiyama and co-workers isolated 518 mg of verucopeptin as a white solid from 26 litres of the fermentation broth. Unlike many other family members, verucopeptin was ineffective as an antibiotic against Gram-positive bacteria, Gram-negative bacteria, and fungi. It did, however, show

potent cytotoxicity, it potently inhibiting the growth of a murine B16-F10 melanoma cell line and a HCT-116 human colon cancer carcinoma cell line (Figure 4).



Cancer Cell Line	IC_{50} / μg/ml
B16-F10 murine melanoma	0.004
P388 murine lymphocytic leukaemia	0.08
Moser human colorectal carcinoma	0.4
HCT-116 human colon carcinoma	0.04

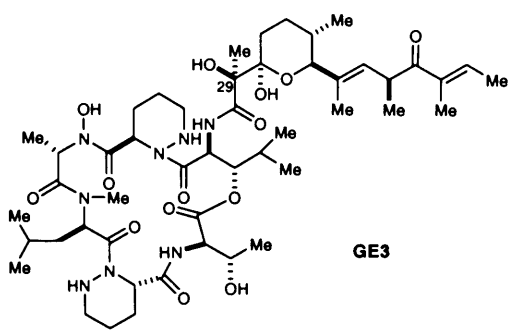
Figure 4 *In vitro* cytotoxicity of verucopeptin

Verucopeptin also showed significant antitumour activity in mice transplanted with B16 melanoma, it exhibiting a T/C of 146~162% by various treatment schedules.[†] Of particular interest was the observation that verucopeptin was even more potent (T/C = 146%) than the known anticancer drug mitomycin C (T/C = 138%) when administered at doses of 0.5 mg/kg/day. At present, the relative and absolute stereochemistry of verucopeptin remains unknown. However it most likely resembles other members of the A83586C/GE3 class, and this was assumed for the purposes of our research work on this molecule.

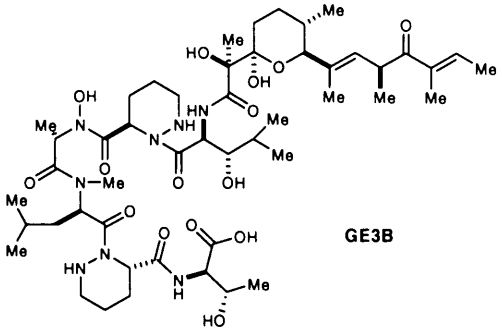
In 1997 the Kyowa Hakko Kogyo team[10,11] isolated the molecule GE3 from culture broths of *Streptomyces* sp.*GE3*, a soil microorganism gathered from the Shimane prefecture of Japan. Structure determination identified the molecule as being a member of the A83586C class. The isolation and purification process yielded 24.7 mg of GE3 and 6 mg of GE3B (a ring opened congener of GE3), from 15 litres of broth. Interestingly GE3 was only weakly active against Gram-positive and Gram-negative bacteria. It did, however, show potent cytotoxicity against various mouse and human tumour cell lines with IC_{50} values that ranged from 6 nM to 16 nM (Figure 5). GE3B showed no antibiotic or cytotoxic effects, signifying the necessity of the 19-membered cyclodepsipeptide ring for the observed biological activity. Perhaps the most important observation that was made on GE3 was the finding that a single intraperitoneal dose of 2 mg/kg to mice

[†] Antitumour effects are often expressed in terms of the ratio (T/C) of the tumour volume of the tested group (T) relative to the tumour volume of the control group (C) to whom no drug is administered

transplanted with PSN-1 human pancreatic carcinoma brought about a significant reduction in tumour growth (T/C = 0.47) without presenting serious toxicity problems. Currently, the latter is an incurable type of human tumour, thus highlighting the importance of GE3 as a potential anticancer therapeutic.



GE3



GE3B

During their endeavours to elucidate the mechanism of action of GE3, the Kyowa Hakko Kogyo team analysed the effect of GE3 on cell cycle progression, and on the expression of the E2F regulated cell cycle related gene, cyclin A. GE3 inhibited the progression of the cell cycle from the G1 to S phase in A431 human lung cancer cells. GE3 also inhibited cyclin A gene expression in Saos-2 cells, and prevented the E2F transcriptional factor from binding to its recognition sequence. It was therefore concluded that GE3 selectively represses the expression of genes essential for the progression of the cell cycle from G1 to S phase and that this is due to its inhibition of E2F transcription factors. Presumably A83586C and citropeptin also prevent E2F transcription factors from activating target genes involved in the cell growth and proliferation.

Although A83586C is a potent antibiotic, GE3 exhibits only very weak antibacterial activity, notwithstanding the fact that the two natural products are identical except at two positions. GE3 possesses an *N*-methyl-D-Leu unit in its cyclodepsipeptide core, whereas A83586C has an *N*-methyl-D-Ala at the same location. The C(29) tertiary alkyl grouping of GE3 is a methyl whereas in A83586C it is an ethyl. Apparently these two structural changes are sufficiently dramatic to alter the antibacterial profile of GE3. It might well be that they are also sufficient to perturb the anticancer profile and mechanism of action.

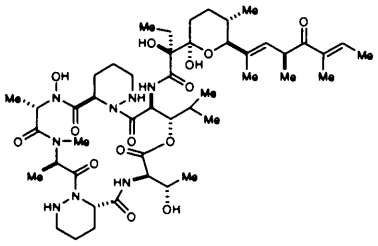
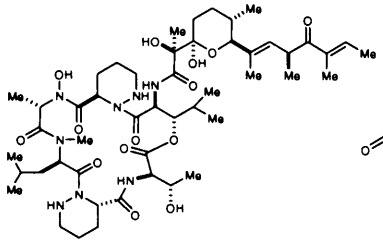
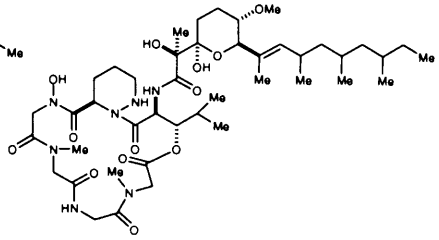
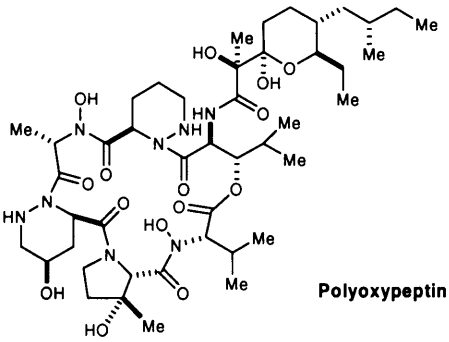
A83586C	GE3	Verucopeptin
		
IC_{50} = 0.0135 μg/ml Vs CCRF-CEM human T-cell leukaemia cell line	IC_{50} = 3.6 nM Vs Saos-2 cells IC_{50} = 6 nM Vs HeLa S3 cells IC_{50} = 16 nM Vs A431 S3 Cells T/C = 0.47 at dose of 2 mg/kg against PSN-1 human pancreatic carcinoma in mice Prevents E2F from binding to their recognition sequence. Inhibits cyclin A gene expression in Saos-2 cells. Inhibits cell cycle progression from G1 to S phase.	IC_{50} = 4 nM Vs B16-F10 Melanoma IC_{50} = 80 nM Vs P388 Leukaemia IC_{50} = 40 nM Vs HCT-116 Cells T/C = 146% at 0.5 mg/kg/day Vs B16 Melanoma in mice (at same dosage Mitomycin C showed T/C = 138%) T/C = 162% at 2 mg/kg/day Vs B16 Melanoma in mice

Figure 5 Summary of the pharmacological properties of A83586C, Verucopeptin and GE3

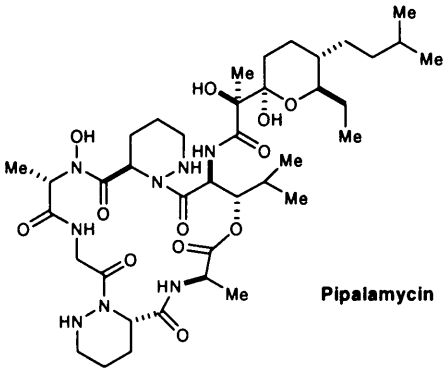
Yet another fascinating member of the A83586C class to have been isolated is polyoxypeptin A. It was obtained from culture broths of the *Streptomyces* species MK498-98F14.[12,13,14] Four litres of broth afforded 330 mg of pure polyoxypeptin A. The structure of this new family member was elucidated by 2D-NMR, degradation studies and X-ray crystallographic analysis. The latter revealed that the cyclodepsipeptide ring of polyoxypeptin A differed from other members of the A83586C/GE3 family in its possession of a (2*S*, 3*R*)-3-hydroxy-3-methylproline unit and a (3*R*, 5*R*)-5-hydroxypiperazic acid fragment in the cyclodepsipeptide sequences. These had not been previously encountered in other family members.



Polyoxypeptin A

Biological testing revealed that polyoxypeptin A was a potent inducer of apoptosis in an apoptosis-resistant cell line of the human pancreatic adenocarcinoma, AsPC-1.[15] The known anticancer agents, adriamycin and vinblastine, also inhibit the growth of these human tumour cells but do not induce apoptosis even at the high concentration of 30 μg/ml. Polyoxypeptin A also decreased the viability in AsPC-1 cells with an ED_{50}[†] value of 0.08 μg/ml. At a concentration of 0.1 μg/ml, it induced nuclear fragmentation and internucleosomal DNA fragmentation after 24 h; behaviour that is characteristic of apoptosis.

The most recent member of the A83586C class to have been identified is pipalamycin,[16] isolated from a culture filtrate of *Streptomyces* sp. ML297-90F8. Pipalamycin induces cell death in an apoptosis-resistant human pancreatic adenocarcinoma AsPC-1 cell line at 0.3 μg/ml over 24-48 hours. These cell deaths are evidenced by the induction of nuclear fragmentation. Pipalamycin also displays antibacterial activity against Gram-positive bacteria such as *Staphylococcus aureus* and *Micrococcus luteus*.



Pipalamycin

It is evident from the biological results that the A83586C class display remarkable antitumour properties; this makes them attractive lead structures for the design and development of new anticancer drugs. Moreover their postulated mode of antitumour activity is of particular interest. The fact that GE3 is claimed to prevent E2F transcription factors from binding to target nucleotide sequences to which they would ordinarily bind is truly fascinating, and deserves further mechanistic clarification, since it has enormous implications for future anticancer drug design against this target. In order to give the reader some insight into the role of E2F transcription factors in controlling cell growth and proliferation, we will now discuss their biological role in more detail and place it in the context of progression through the cell cycle.

[†] Effective Dose; amount of material required to produce a specified effect in 50% of the test population

CHAPTER 2 The E2F Transcription Factors

The cell cycle is the process used by all eukaryotic cells to control growth and division. It regulates the replication of the genome and the subsequent segregation of chromosomes into daughter cells. There are four broad phases of the cell cycle (Figure 1). The G1 (Gap 1) phase is characterized by gene expression and protein synthesis, and is regulated primarily by extracellular stimuli such as mitogens and adhesion. During G1 the cell produces all the necessary proteins for DNA synthesis. This enables the cell to enter the S phase, where DNA replication occurs, allowing the cell to divide into two daughter cells, each having a complete copy of DNA. Before this occurs the cell must enter a third phase known as G2 (Gap 2 phase). During G2 the cell undergoes growth and protein synthesis, priming it to divide. Once complete, the cell enters the fourth and final phase of cell cycle: the M (mitosis) phase. In M phase the chromosomes segregate into two daughter cells. After mitogenic signalling ceases, cells can exit the cell cycle for a period and enter a quiescent state known as G0. Alternatively they terminally differentiate into cells that will not divide again, and instead undergo morphological development in order to carry out the various specialized functions of individual tissues.

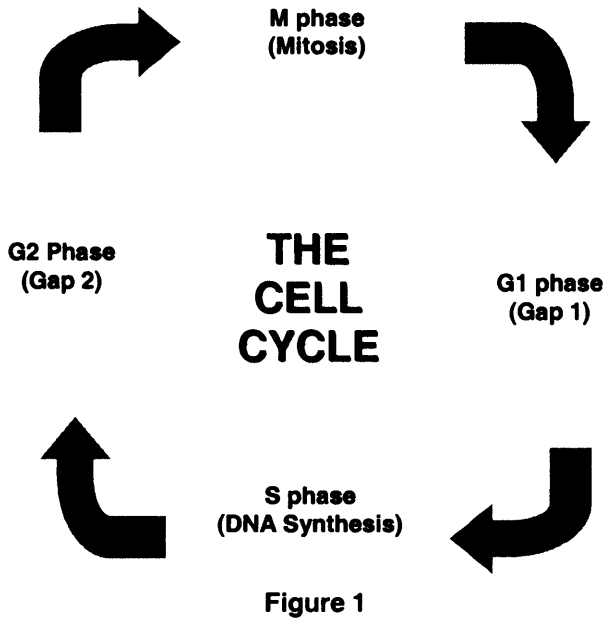


Figure 1

The ordered progression through the cell cycle is dependent on various checkpoint control mechanisms which are regulated in mammalian cells by E2F transcription factors. E2F transcription factors, along with other transcriptional activators execute a complex regulatory cascade of gene expression, which controls cellular DNA synthesis and cell proliferation. They also

play a major role in cell-cycle arrest and apoptosis; an essential process if a major malfunction or DNA damage is encountered during the cell cycle. It is now widely believed that E2F transcription factors and other proteins involved in their activity constitute a genetic pathway that is most probably the most frequently altered in human cancer.

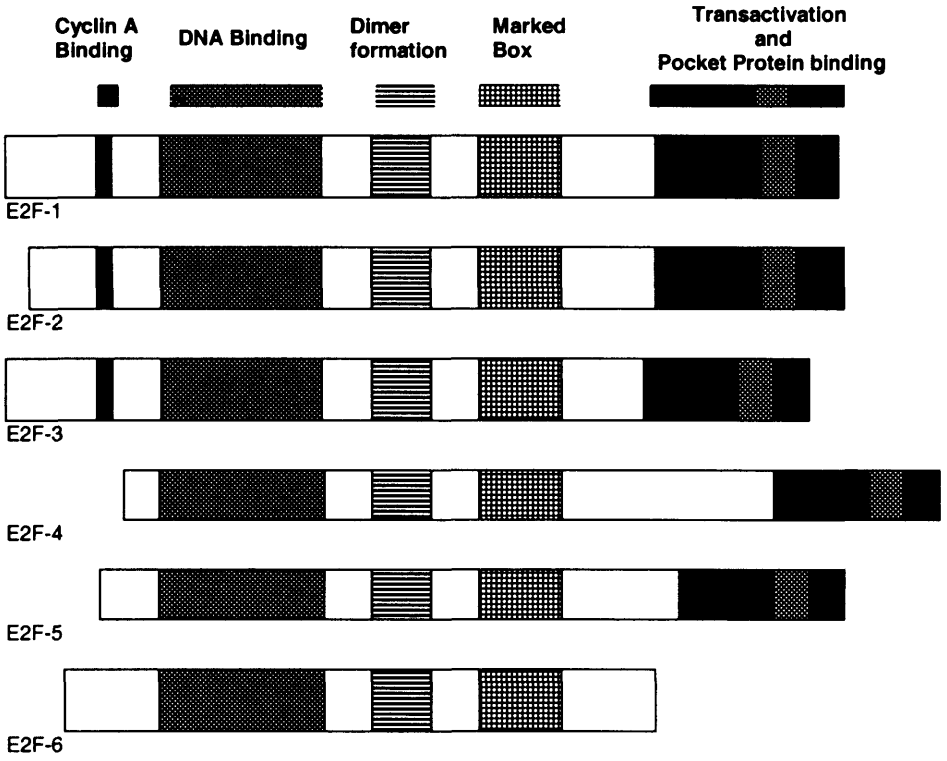


Figure 2 The E2F family of transcription factors[19,20]

E2F transcription factors[17,18,19,20] are heterodimeric proteins comprised of one E2F protein and one DP protein. Currently six E2F proteins (E2Fs 1-6),[21,22] have been identified. Each of these can bind to one of two DP proteins, DP1 and DP2. E2Fs 1-6 are divided into three groups based upon their differing homology (Figure 2). All members contain a DNA binding domain within their *N*-termini[21] and next to this is a dimerization domain through which the E2F/DP heterodimers form. There is also sequence homology within the marked box region of all family members although its function has yet to be defined. All E2Fs with the exception of E2F-6 have *C*-terminal transactivation domains with a pocket protein-binding domain embedded within. This region is

responsible for the regulation of E2Fs 1-5 through their binding to the retinoblastoma (Rb) family of tumour suppressors, also known as the pocket proteins. The fact that E2F-6 lacks this transactivation domain is consistent with its function as a repressor of E2F site-dependent transcription that is independent of pocket protein binding.[23] E2Fs -1 to -3 have further conserved regions *N*-terminal to the DNA binding domain which are not observed in the other three family members. This domain is responsible for binding to the cyclin A protein.[24, 25]

A functional E2F transcription factor requires the formation of a heterodimer from one E2F protein and one DP protein.[26,27,28] E2F/DP heterodimers recognize and bind to specific DNA sequences contained within their target genes to regulate their expression. The key recognition sequences are 5' -TTSSCGC -3' (where S = C or G).[29] These are located in the promoter regions of many genes important for satisfactory progression through the mammalian cell cycle.[30] Target genes for E2F transcription factors include cellular oncogenes such as IGF-1, c-*myc* and B-*myb*; tumour suppressor genes such as Rb and p107; rate-limiting regulators of DNA synthesis such as DNA polymerase α, dihydrofolate reductase, thymidine kinase and topoisomerase I; and genes that encode for proteins essential for progression through the cell cycle such as cyclin A, cyclin D1 and cyclin E.[31-37] E2F transcription factors regulate the levels of these genes in a cell cycle dependent fashion, ensuring that the appropriate genes are maximally expressed in the late G1 and early S phases of the cell cycle.

E2F / DP heterodimers are regulated through their association with members of the Rb family of tumour suppressor proteins (Figure 3). There are three members of this family, pRb, p107 and p130 which are collectively known as the pocket-proteins.[38] E2Fs -1 to -3 associate exclusively with pRb but not with p107 or p130;[39] E2F4 associates with all three Rb proteins;[40] while E2F5 associates only with the p130 protein.[41] The pocket proteins form complexes with the E2F / DP heterodimers that inhibit their transcriptional activation capacity. This is certainly the case during the early- to mid-G1 phase of the cell cycle and in non-cycling G0 cells, where the E2F / DP dimer is kept in its quiescent state. As the cell approaches the late G1 phase of the cell cycle,

the Rb family of proteins become phosphorylated and as a consequence the free transcriptionally active E2F / DP complexes[42] are primed. It is only the hypophosphorylated forms of pocket proteins that are functionally active in binding to and inactivating E2F / DP complexes. When decomplexed from the pocket proteins, E2F / DP transcriptional factors are able to switch on their target gene promoters and activate their transcription. This results in the synthesis of proteins required for the cell to make the transition from the G1 to the S phase of the cell cycle and hence allow cell proliferation. In mid S phase, after the E2Fs have activated their target gene sets, their activity is inhibited by phosphorylation.[24] Moreover, during M phase and into the next cell cycle, the Rb proteins become hypophosphorylated and are again able to bind and inactivate the E2F / DP heterodimers[43] and prevent them from activating target gene expression.

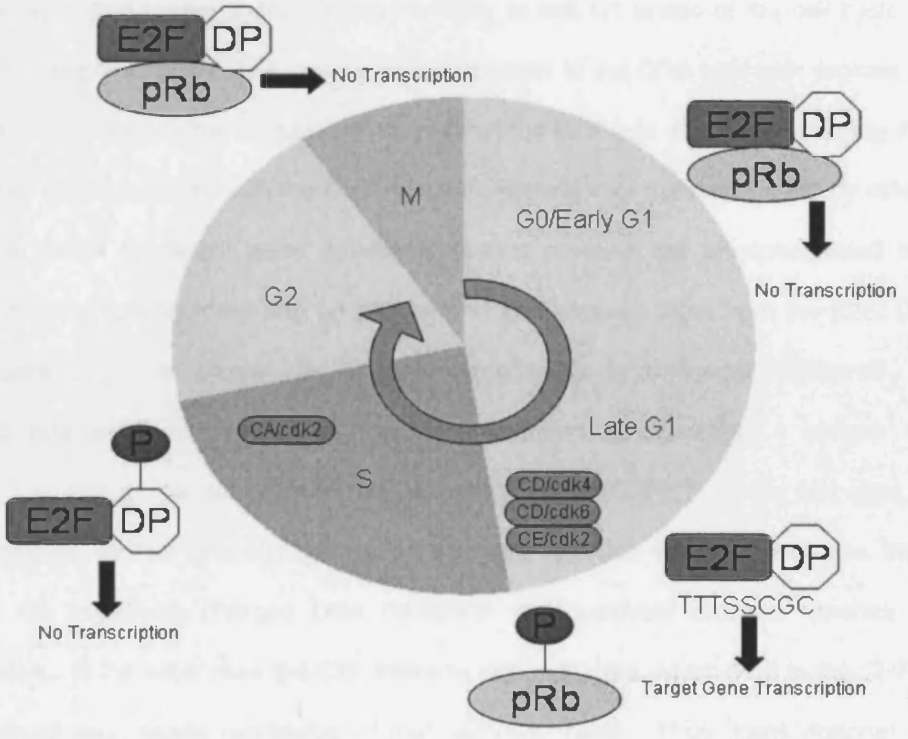


Figure 3 The regulation of E2F transcription factors

Pocket protein phosphorylation is controlled by various combinations of cellular kinases formed from the cyclins and cyclin-dependent kinases (CDKs). Cyclin D/cdk4, cyclin D/cdk6 and cyclin E/cdk2 are all known to cause the phosphorylation of Rb proteins[38] allowing dissociation of the E2F / DP dimer from the Rb protein to bring about transcriptional activation, as stated above. Cyclin A/cdk2[24] phosphorylates the DP subunit of the dimer during S phase, and this phosphorylated form of the E2F / DP dimer is no longer able to bind to the DNA consensus sequence in the promoter region of target genes. The cyclin / cdk proteins are further controlled by the action of CDK inhibitors (CDKI). Members of this group include p21, p27 and p19 which all result in cyclin / cdk activity[44] being downregulated.

E2F transcription factors not only function as transcriptional activators, they also act as transcriptional repressors, preventing the expression of some target genes.[45,46] The complex responsible for transcriptional repression is the E2F / DP dimer in association with the Rb protein; it is this complex that predominates during the early to mid G1 phase of the cell cycle, and in non-cycling G0 cells (Figure 4). The binding of this complex to the DNA promoter regions prevents the activation of target gene transcription. It seems that the E2F acts as a tether to bring the bound Rb protein into close proximity with the DNA, blocking activation of the target gene by other proteins.[47] As in the model for target gene activation, pocket proteins are phosphorylated by cyclin/cdk kinases as cells approach the late G1 phase, and this releases them from the E2F/ DP dimer. In the absence of pocket protein, target gene transcription is no longer repressed. E2F / pRb mediated repression is believed to involve recruitment of the class 1 histone deacetylase, HDAC1,[48] as well as the cell cycle genes homology region (CHR).[49] In the first case, the HDAC1 *N*-deacetylates various *N*-acetyl groups on the core histones which strengthens the interaction between the negatively charged DNA backbone and positively charged histones to suppress transcription. In the latter case the E2F interacts with cofactors, which bind to the CHR site, and in an undefined way, cause repression of that particular gene. Thus, transcriptional activation of target genes by free E2F / DP dimers occurs mainly in late G1 and early S phases of the cell cycle, whereas repression occurs when the dimer is bound to the Rb protein which occurs in the cell cycle

phases other than G1 and early S. Whether a target gene is repressed or transactivated appears to be dependent on tissue specificity, cell cycle position, and on the level of transcription factor expression within a given cell.

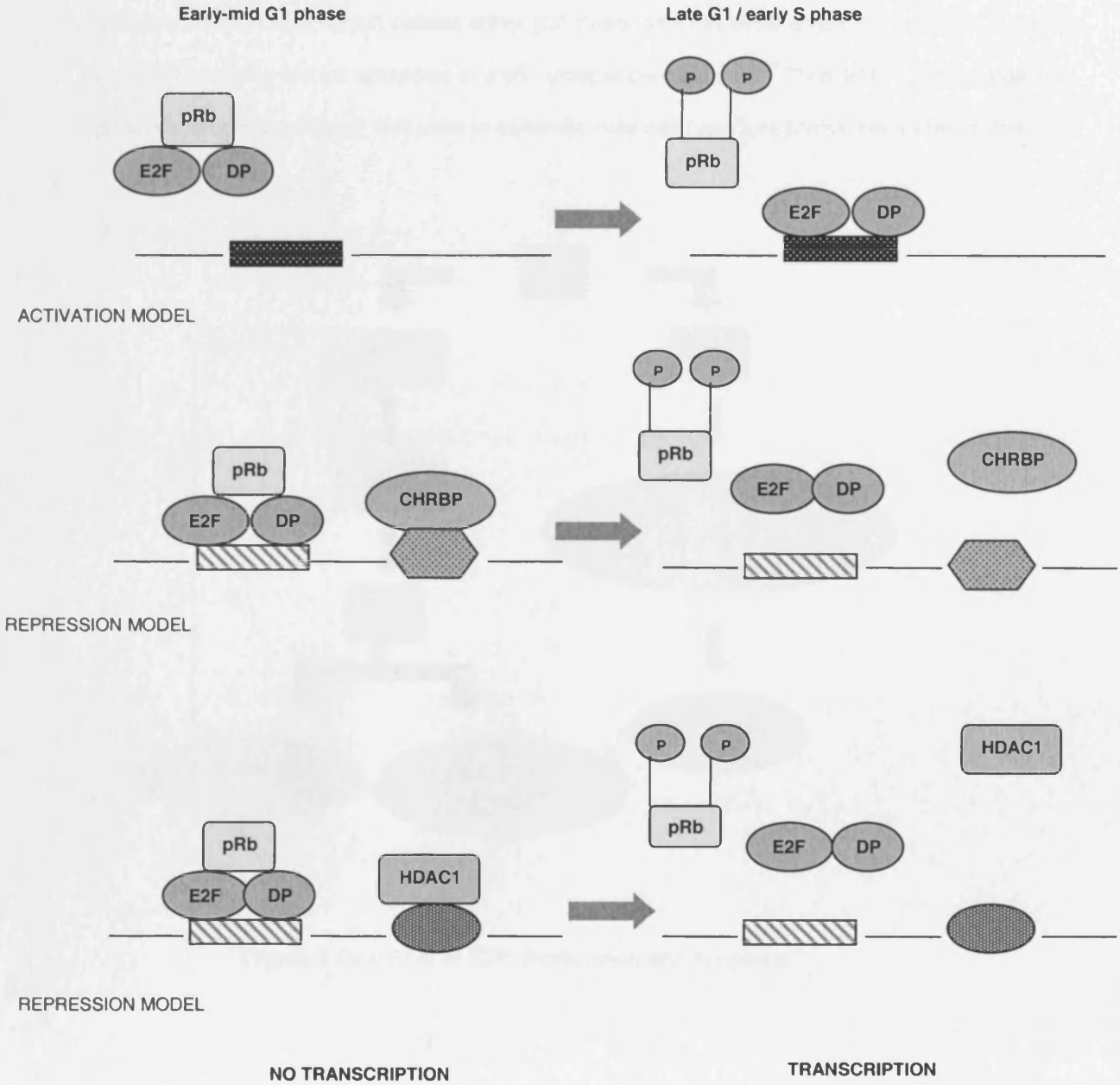


Figure 4 Activation and Repression by E2F[17]

Besides the role the E2F / Rb pathway plays in controlling cell proliferation, it is also clear that it can determine cell fate by inducing apoptosis (programmed cell death).[50] E2F -1 can cause cells to undergo apoptosis when overexpressed.[51] It is unique in this respect as it is the only family member that can induce both proliferation and apoptosis.[52,53] The E2F-1 protein is believed to promote apoptosis through transcriptional activation of the $p19^{ARF}$ gene, which in turn stabilises the p53 tumour suppressor, which causes either p53 dependant cell cycle arrest or apoptosis[54] (Figure 5). E2F-1 can also induce apoptosis in a p53 independent manner.[55] Thus E2F-1 participates in a protective apoptotic pathway that aims to eliminate cells that have lost normal cell cycle control.

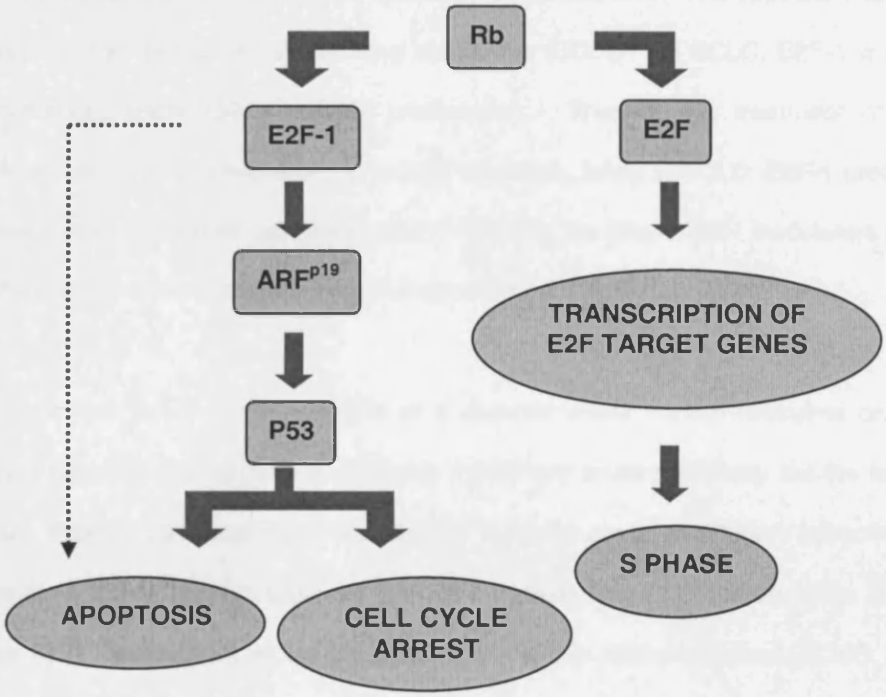


Figure 5 Dual Role of E2F: Proliferation and Apoptosis[54]

In normal cells a precise balance of proliferation versus apoptosis must be maintained and this appears to be under the control of E2F-1.[56] Disruption of this delicate equilibrium can result in an aberration of normal cell growth. In cells where E2F-1 contributes mainly to proliferation and p53 levels are low, overexpression of E2F-1 results in tumours, whereas loss of E2F-1 leads to tissue atrophy. Thus E2F-1 can function as an oncogene. In the case where E2F-1 promotes apoptosis, its overexpression leads to tissue atrophy and its loss leads to the formation of tumours. Hence E2F-1 can act as a tumour suppressor. This dual mode of action of E2F-1 as an oncogene or as a tumour suppressor has been highlighted by a recent study of E2F-1 expression in human lung tumours.[57] It was shown that in non-small cell lung carcinoma (NSCLC), inactivation of E2F-1 detrimentally contributes to defective apoptosis of tumour cells. The opposite was seen in the most aggressive lung tumour, small cell lung carcinoma (SCLC). In SCLC, E2F-1 is up-regulated and this promotes uncontrolled cellular proliferation. Thus in the treatment of NSCLC, E2F-1 expression should be upregulated to induce apoptosis, while in SCLC, E2F-1 production should be downregulated to prevent cell proliferation. Thus, in the future, E2F modulators might have to be used in a highly selective fashion against tumours.

Cancer is the prime example of a disease where normal restraints on cell proliferation abate. Therapies that target the cell cycle machinery could potentially aid the future treatment of cancer. Protein components of the Rb/E2F pathway are a particularly attractive target as they constitute a linear genetic pathway that is the most frequently deregulated in human cancers (Figure 6).[58,59] Mutations in the Rb genes are found in retinoblastomas, SCLC, NSCLC, bladder, breast and prostrate carcinomas.[60] Mutations in the cyclin and cdk genes are frequently associated with head and neck squamous cell carcinomas, oesophageal carcinomas, bladder cancers and ductal breast cancers.[61] Increased expression of cyclin D has been implicated in the onset of breast cancer (which accounts for 20% of all female cancer deaths in UK) and colon cancer. Up to 50% of breast carcinoma cells show increased expression of the cyclin D1 gene.[62] Cyclin D1 expression, which upregulates E2F, is regulated by β-catenin in colon carcinoma cells. Mutations in the p16 CDKI gene are the second most common mutation in human cancer (20 to

40% of human cancers)[63] next to mutations in the p53 tumour suppressor protein. Since the activation of E2F activity is the consequence of deregulation of the Rb pathway, irrespective of the nature of the mutation, controlling the activity of E2F proteins could offer new hope in the treatment of cancer.

It is now widely thought that molecules with the capacity to target the cell cycle machinery might be able to prevent vascular occlusions from developing after vein graft operations,[64,65] where uncontrolled E2F activity is frequently responsible for causing the occlusion around the site of tissue injury. Atherosclerosis and restenosis are responsible for 50% of all deaths in the Western world and are the major causes of heart attack, stroke and extreme gangrene.[66] Restenosis can occur one to six months following unsuccessful treatment of atherosclerotic plaque via procedures such as angioplasty.[67] Human bypass vein graft failures are also common, and are caused by the formation of an abnormal inner lining (neointimal hyperplasia) that is susceptible to atherosclerosis. The major mechanism responsible for these post-operative complications is the hyperproliferation of vascular smooth muscle cells (VSMC). In a recent study,[68] it was observed that decoy oligonucleotides specific for E2F-1, can prevent the upregulation of genes responsible for driving the cell through DNA synthesis and mitosis. It was little surprise therefore to find that they could block VSMC proliferation and neointimal hyperplasia in injured vessels. In this trial the occurrence of primary graft failure was compared between patients who received and who did not receive the E2F decoy treatment. Whilst failures occurred among the untreated group throughout the 12-month follow up period, no failures were observed in the E2F decoy treated graft group beyond the first 6 months. Hence molecules that target the cell cycle components, in particular E2F transcription factors, might therefore aid the development of future medicines effective in diseases that are characterised by aberrant cellular proliferation.

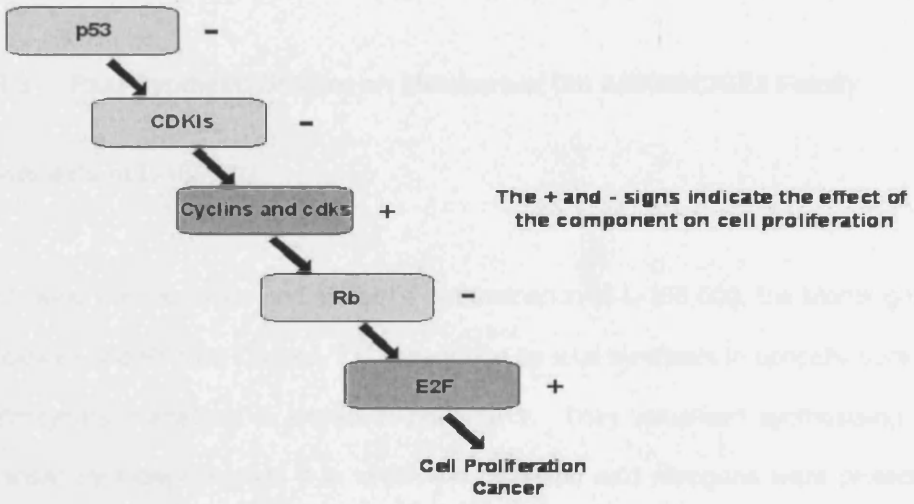


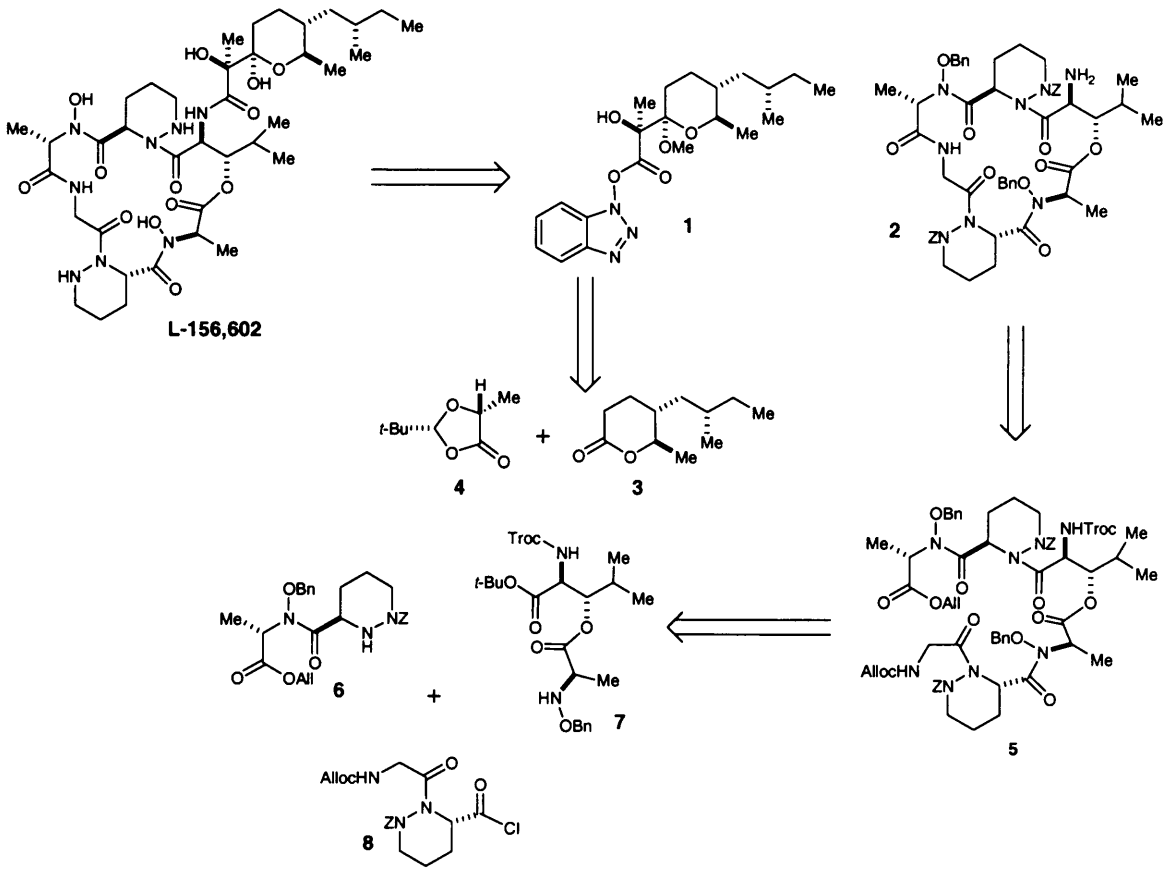
Figure 6 The E2F genetic pathway[17]

In conclusion the E2F/Rb signalling pathway is an important and critical regulator of cellular proliferation that is disrupted in virtually all human cancers. It will thus be appreciated that small molecule drugs that can counteract defects in this pathway might potentially impact future cancer therapy and cardiovascular disease. The fact that GE3 was claimed to prevent E2F transcription factors from binding to target DNA nucleotide sequences that possess the key consensus sequence, 5' -TTSSCGC -3' (where S = C or G), suggests that GE3 might be acting as an E2F inhibitor, especially when considered alongside its reported effects on cyclin A gene expression in Saos-2 cells and its inhibition of cell cycle progression from the G1 to S-phase in A431 human lung cancer cells. Given its extraordinary potency against a wide range of human tumour cell lines, and its good *in vivo* activity against xenografted solid human tumours in mice when administered at low doses, GE3 is therefore an exciting new lead in the E2F inhibitor design area. It is certainly the most potent E2F inhibitor to have so far been discovered, and as such, synthetic interest in the preparation of this and related molecules is likely to be high in the coming years. In the next chapter, we will discuss synthetic work on this class of natural products, which will serve as the prelude to us describing our own synthetic efforts in the A83586C/GE3/verucopeptin analogue area.

CHAPTER 3 Past Synthetic Studies on Members of the A83586C/GE3 Family

3.1 Synthesis of L-156,602

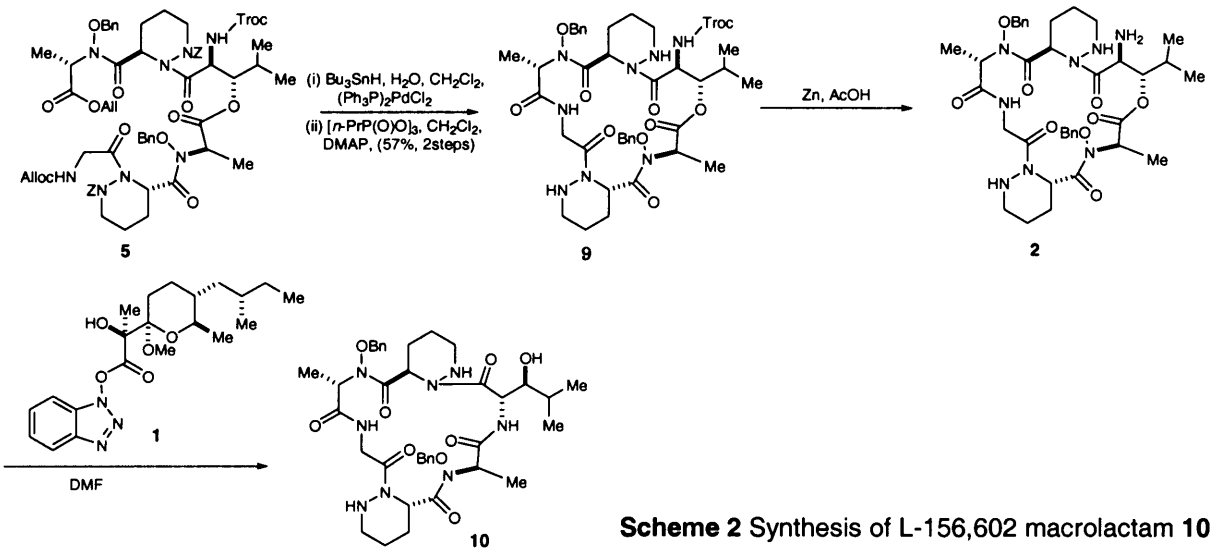
Following their isolation and structure determination of L-156,602, the Merck group led by Charles Caldwell and Phillipe Durette, [69, 70] attempted its total synthesis in optically pure form. The original retrosynthetic analysis is shown in Scheme 1. They visualised synthesising a partially protected linear cyclodepsipeptide **2** in which the piperazic acid nitrogens were protected with Z (benzyoxycarbonyl) groups, the NOH groups were protected as *O*-benzyl ethers, and the anomeric hydroxyl was masked as a methyl glycoside.



Scheme 1 Caldwell and Durette's first retrosynthetic analysis of L-156,602

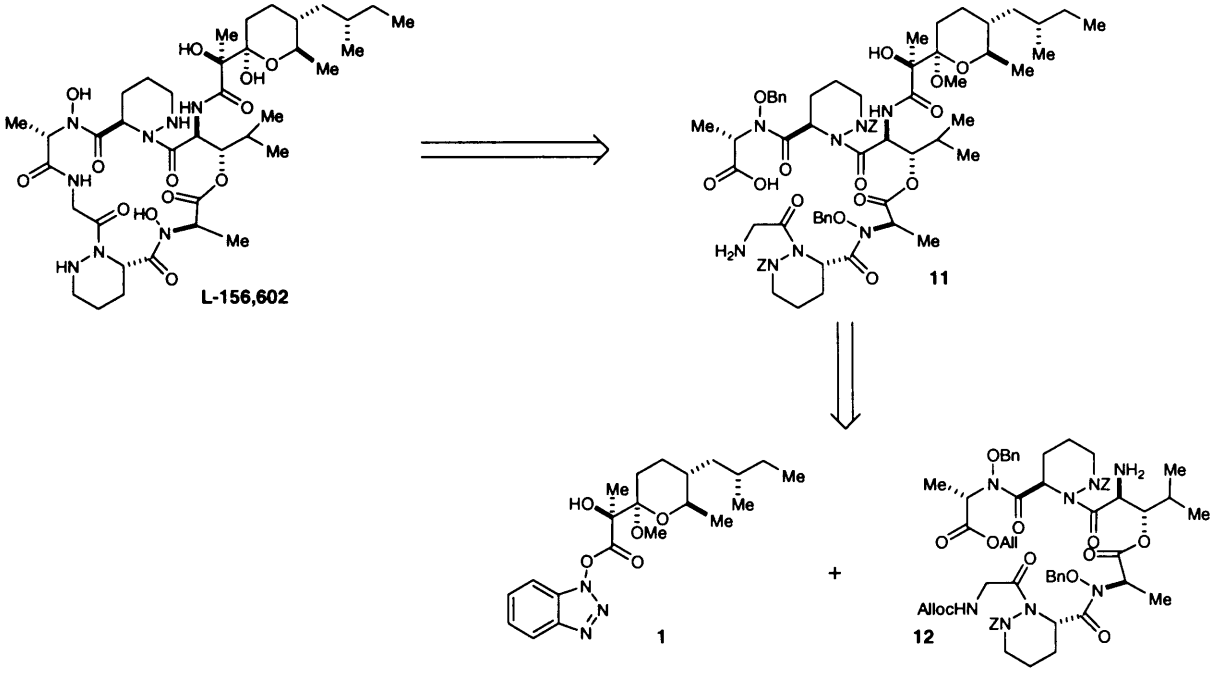
Linear hexadepsipeptide **5** was selected for ring closure the macrolactamisation could be effected between the glycine and *N*-benzyloxy-L-alanine residues. This ring closure was chosen because it allowed the most nucleophilic and least sterically hindered nitrogen to be used for the attack on the activated carbonyl group. Their assembly strategy for the linear hexapeptide **5** was based upon successive [2+2+2]-fragment condensation of the protected dipeptides **6**, **7**, and **8**. The quaternary stereocentre at C(29) in activated ester **1** would be constructed via a Seebach enantioselective Claisen condensation reaction,[71] and a Frater-Seebach alkylation was envisaged for accessing lactone **3**.

In their first attempt at synthesising L-156,602 Caldwell and Durette successfully constructed linear hexadepsipeptide **5** and activated ester **1** (their total syntheses will be discussed below). The *N*-terminal Alloc and *C*-terminal *O*-allyl ester groups of **5** were cleaved in a single step by palladium-catalysed hydrogenolysis with Bu_3SnH, and the hexadepsipeptide cyclised via a mixed phosphonic anhydride to give protected cyclic hexadepsipeptide **9** (Scheme 2). The Troc group was cleaved from the 3-hydroxy-Leu of **9** residue with Zn/AcOH and the resulting crude amine **2** reacted with activated ester **1**. Unfortunately, none of the desired amide was produced; in fact the only product isolated was the ring-contracted macrolactam **10** resulting from *O*- to *N*-acyl shift. Consequently resort had to be made to introducing the side chain at the linear hexadepsipeptide stage.



Scheme 2 Synthesis of L-156,602 macrolactam **10**

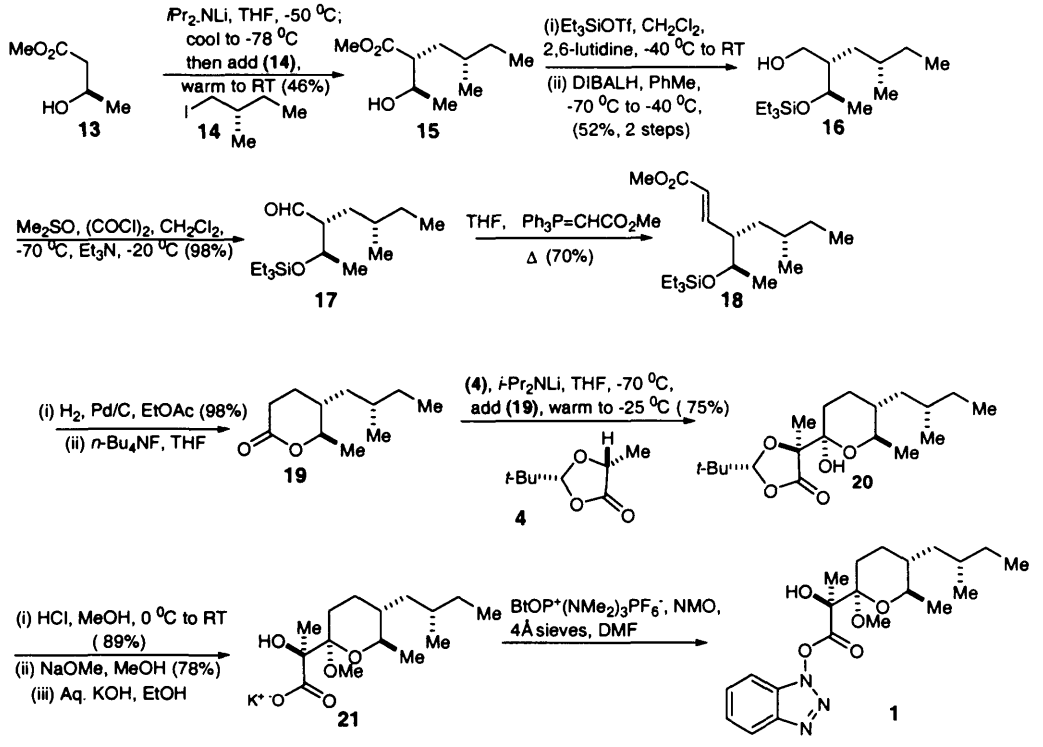
As a consequence they revised their retrosynthetic strategy to that shown in Scheme 3. The fragments required for the synthesis of activated ester **1** and the linear hexadepsipeptide **12** were identical to the first route. The only difference was the execution of the coupling reaction at an earlier stage. It was thought that to prevent the offending *O*- to *N*-acyl shift, the 3-hydroxy-Leu residue of the linear hexadepsipeptide **12** had to be capped with the pyran fragment prior to macrolactamisation. This would afford the pre-macrolactamisation precursor **11** which, following deprotection of the terminal protecting groups, would undergo macrolactamisation to afford the natural product without this problem occuring.



Scheme 3 Caldwell and Durette's revised retrosynthetic analysis of L-156,602

The synthesis of the tetrahydropyran sector of L-156,602 (Scheme 4) began with a diastereoselective Frater-Seebach alkylation reaction[72] between the enolate derived from methyl (*R*)-3-hydroxybutanoate **13** and (*S*)-1-iodo-2-methylbutane **14**, which resulted in alcohol **15**. Lactone **19** was obtained from **15** in a further six steps which commenced with protection of its hydroxyl as a triethylsilyl ether and reduction of the methyl ester with DIBAL to afford alcohol **16**. Alcohol **16** was then oxidised under Swern conditions, and aldehyde **17** condensed with

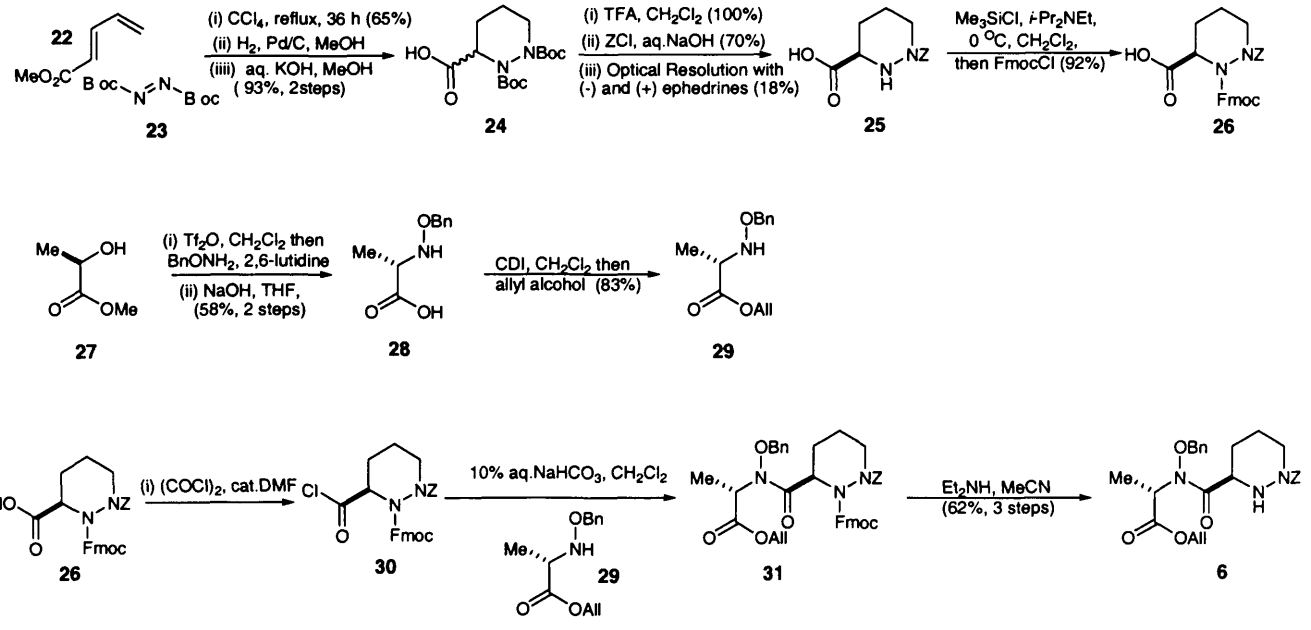
carboxymethyltriphenylphosphorane. The resulting (*E*)-enoate **18** was then hydrogenated over Pd/C and *O*-desilylation used to bring about ring closure to the lactone **19**.



Scheme 4 Synthesis of L-156,602 pyran **1**

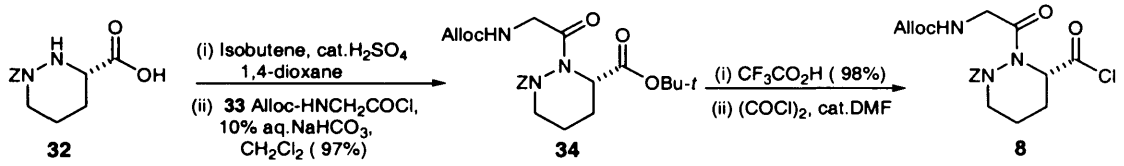
Lactone **19** underwent a highly diastereoselective Claisen condensation with the lithium enolate derived from (2*R*, 5*R*)-2-*t*-butyl-5-methyl-1,3-dioxolan-4-one **4**. The desired product **20** was obtained as a single diastereoisomer in 75% yield. A Fischer glycosidation was next used to block the hemiketal functionality so as to prevent a retro-Claisen reaction from occurring during the base assisted cleavage of the lactone in **20**. Unfortunately saponification of the glycosidated lactone required extended reaction times. Because the product acid decomposed when isolated, transesterification was performed with NaOMe in methanol and the methyl ester was saponified with aqueous KOH in ethanol to give the more stable potassium carboxylate salt. When treated with Castro's BOP reagent[73] the salt gave activated ester **1**.

The [2+2+2]-fragment condensation strategy proposed for assembly of linear protected hexadepsipeptide **5** required the preparation of protected dipeptides **6** and **8** (Schemes 5 and 6) and depsipeptide **7** (Scheme 7 and 8). The enantiomeric *Z*-protected piperazic acids, **20** (3*R*) and **25** (3*S*), were prepared via a Diels-Alder cycloaddition reaction between di-*t*-butylazodicarboxylate **23** and methyl 2, 4-pentadienoate **22** in hot CCl_4 (Scheme 5). Hydrogenation of the double bond in the cyclohexene ring and saponification yielded racemic acid **24** in 93% overall yield. Quantitative deprotection of the Boc groups from **24** with trifluoroacetic acid and *Z* group protection of the *N*(1)-atom, yielded racemic *N*(1)-*Z*-piperazic acid which was resolved with (+)- and (-)- ephedrines.[74] This procedure provided the (3*R*)-*Z*-piperazic acid **25** in 18% yield, and the (3*S*)-*Z*-piperazic acid **32** in 15% yield. The *N*(2)-atom of **25** was protected with an Fmoc group, with the acid being temporarily protected during this reaction as a trimethylsilyl ester, which hydrolysed upon aqueous workup, to give **26**. The protected dipeptide **6** was formed via a Carpino biphasic coupling[75] between **30** and the partially protected hydroxamic acid ester **29**. Hydroxamic acid ester **29** was synthesised in three steps from commercially available (*R*)-methyl lactate **27**. Removal of the Fmoc urethane was performed using diethylamine in acetonitrile to access the target dipeptide **6** in 62% yield over three steps.



Scheme 5 Synthesis of L-156,602 depsipeptide **6**

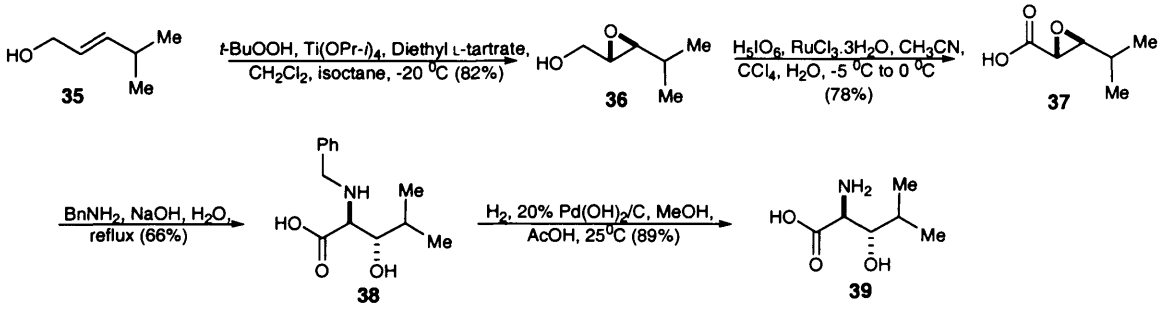
The same Carpino coupling technology was employed for the preparation of **34** (Scheme 6). Thus (3*S*)-*Z*-piperazic acid **32** was protected as a *t*-butyl ester and this coupled to the acid chloride of *N*-Alloc-glycine **33** to furnish dipeptide **34**. This was converted into acid chloride **8** in a further two steps, by acid cleavage of the *t*-butyl ester and chlorination with oxalyl chloride.



32 **34** **8**

Scheme 6 Synthesis of L-156,602 depsipeptide **8**

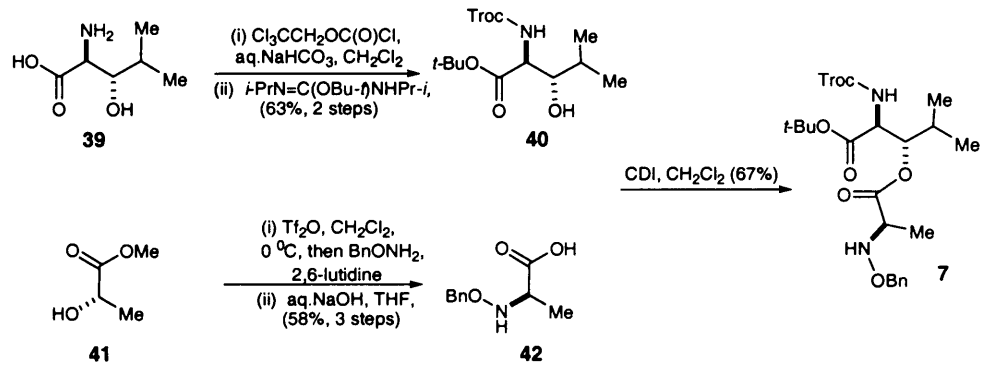
The synthesis of depsipeptide **7** required construction of (2*S*, 3*S*)-3-hydroxyleucine[76] **39** in enantiomerically pure form. Caldwell developed an efficient route to this compound (Scheme 7). The starting point was a Sharpless asymmetric epoxidation on allylic alcohol **35**. Subsequent oxidation of the epoxy-alcohol with periodic acid furnished epoxy-acid **37**. Regioselective ring-opening with benzylamine, followed by hydrogenation of the *N*-benzyl group afforded (2*S*, 3*S*)-3-hydroxyleucine **39**.



35 **36** **37** **38** **39**

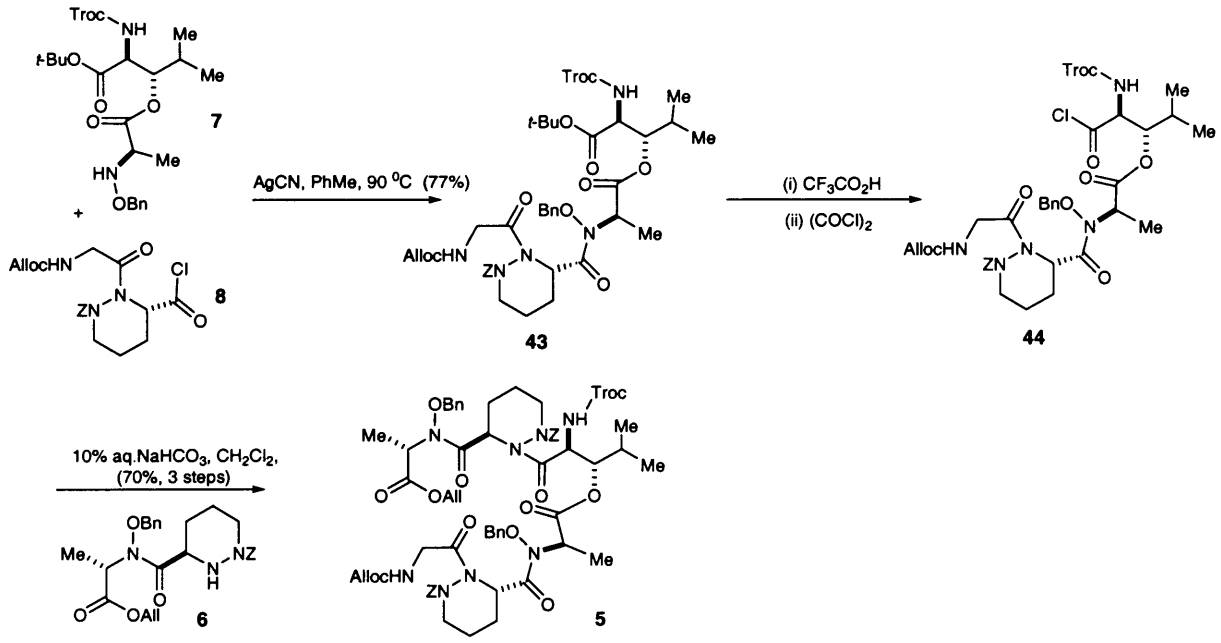
Scheme 7 Caldwell's route of (2*S*, 3*S*)-3-hydroxyleucine **39**

With completion of the (2*S*, 3*S*)-3-hydroxyleucine **39**, depsipeptide **7** was synthesised as depicted in Scheme 8. After regioselective protection of (2*S*, 3*S*)-3-hydroxyleucine **39**, **40** was coupled to the *N*-hydroxybenzyl-(*R*)-Ala residue **42**, utilising 1,1'-carbonyldiimidazole to furnish didepsipeptide **7**.



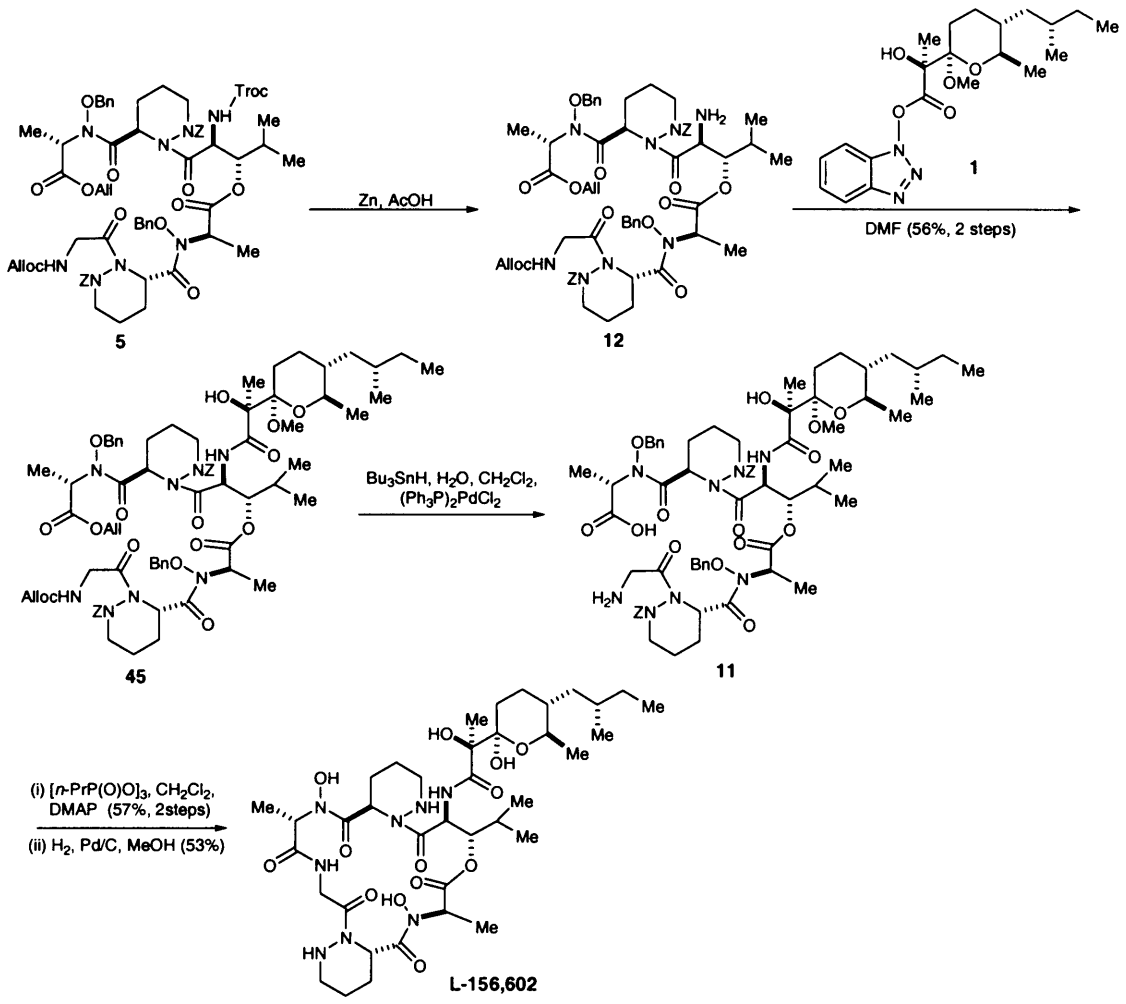
Scheme 8 Synthesis of L-156,602 ester **7**

The protected linear hexapeptide **5** was assembled by a [2+2+2] fragment condensation strategy (Scheme 9). The first [2+2] coupling involved acid chloride **8** and protected hydroxylamine **7** and used silver cyanide amidation technology to obtain peptide **43**. Following cleavage of the *t*-butyl ester with TFA, the free acid was converted to the acid chloride **44** and this employed for a [4+2] coupling with dipeptide **6** using Carpino's biphasic coupling technology to furnish linear protected hexadepsipeptide **5**.



Scheme 9 Synthesis of macrolactam precursor **5**

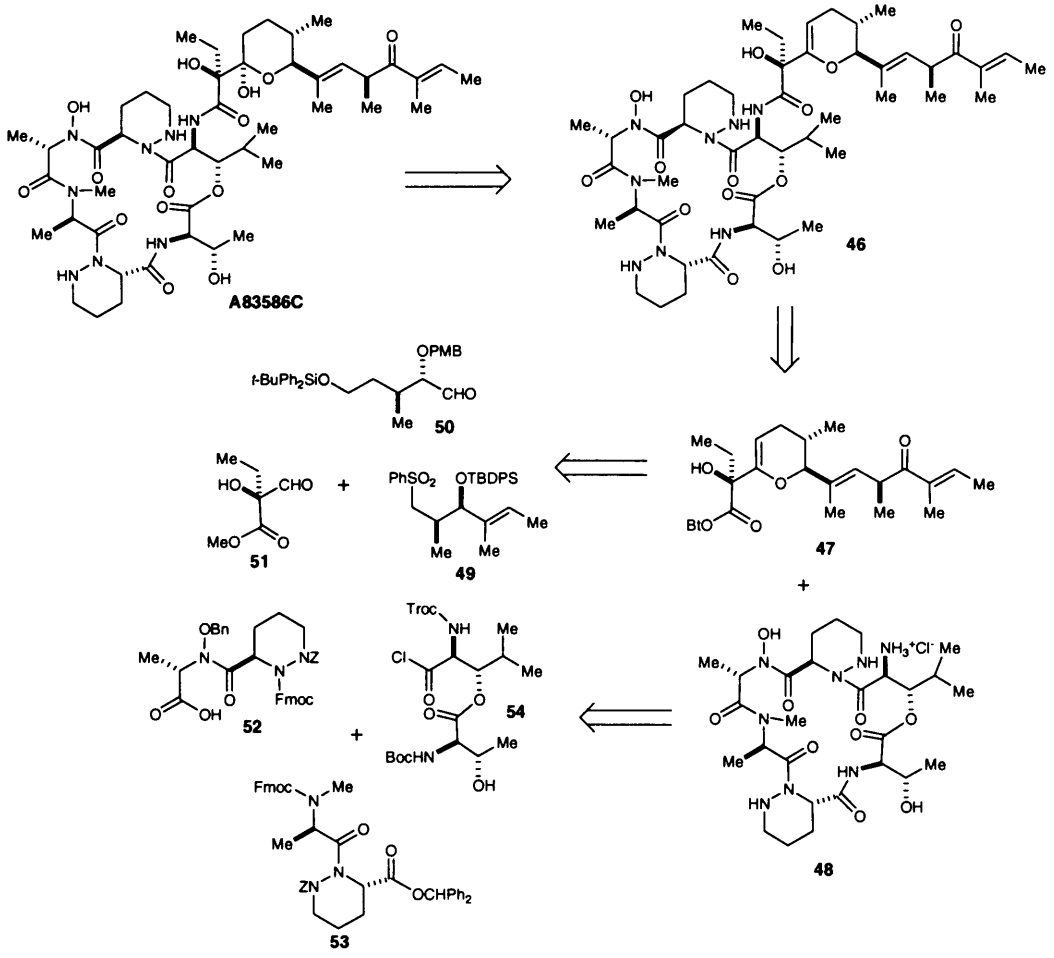
The total synthesis was eventually completed as illustrated in Scheme 10. The Troc group was excised from **5** with zinc dust and the resulting amine **12** coupled with the activated ester **1** to give **45** in 56% yield for the 2 steps. The key macrolactamisation was attempted after the terminal allyl-protecting groups were removed from **45** with Pd(0) and tri-n-butylstannane.[77] This yielded **11** whose 19-membered ring was closed with *n*-propane phosphonic anhydride[78] and DMAP. The synthesis of L-156,602 by Caldwell and Durette was completed by hydrogenolytic removal of the *Z*- and *O*-benzyl protecting groups.



Scheme 10 Completion of the total synthesis of L-156,602

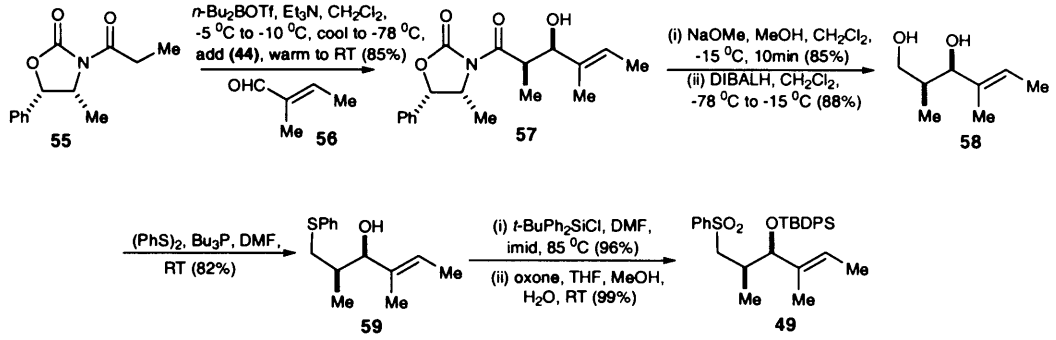
3.2 Synthesis of A83586C

In 1997, Hale and coworkers reported the first asymmetric total synthesis of A83586C.[79] Their strategy (Scheme 11) featured the chemoselective union of the fully elaborated hydroxyl-benzotriazole activated ester **47** with the hydrochloride salt of cyclodepsipeptide **48**. It was felt that the product glycal **46** could be coaxed into undergoing a chemoselective hydration under mild acid conditions to afford the natural product. The route would specifically avoid the use of heteroatom protecting groups at the final stages, which was considered essential given the target's lability towards bases, acids, oxidants, reductants and a variety of strong nucleophiles.



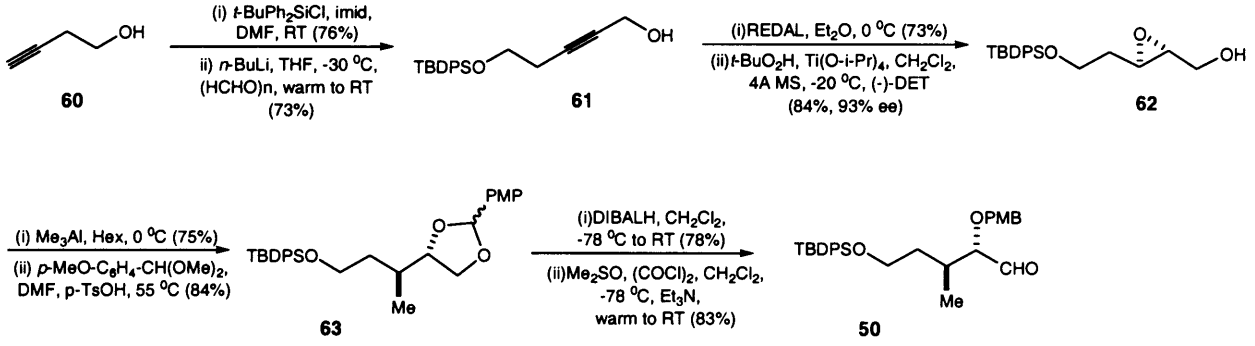
Scheme 11 Hale's retrosynthetic analysis of A83586C

The synthesis of pyran **47** revolved around the construction of intermediates **49**, **50**, and **51**. Sulfone **49** was assembled in six steps (Scheme 12) from the enantiopure propionamide **55** via an Evans asymmetric aldol reaction[80] which installed the two *syn* stereocentres and the (*E*)-trisubstituted alkene. The chiral oxazolidinone was cleaved in two steps by transesterification and methyl ester reduction, which collectively provided the 1,3-diol **58**. Selective thioetherification with tributylphosphine and phenyldisulfide followed by *O*-silylation and Trost-Curran oxidation[81] with Oxone® furnished the required sulfone **49** in an overall yield of 50%.



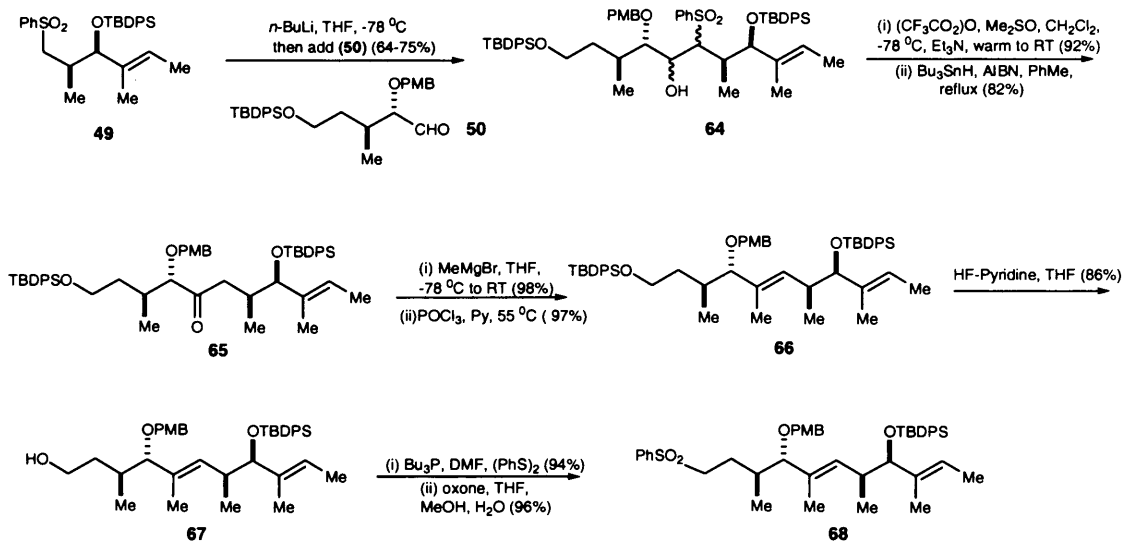
Scheme 12 Synthesis of phenylsulfone **49**

Intermediate **50** (Scheme 13) has an *anti*-relationship between its two adjacent stereocentres. This was installed by a chelation controlled epoxide ring opening reaction on the chiral 2,3-epoxy alcohol[82] **62** with trimethylaluminium, which proceeded with 20:1 C3/C2 selectivity[83]. The main product was then protected as a *p*-methoxybenzylidene acetal and **63** reductively cleaved[84] to selectively protect the secondary alcohol as a *p*-methoxybenzyl ether. The final step to aldehyde **50** was Swern oxidation of the primary alcohol.



Scheme 13 Synthesis of aldehyde **50**

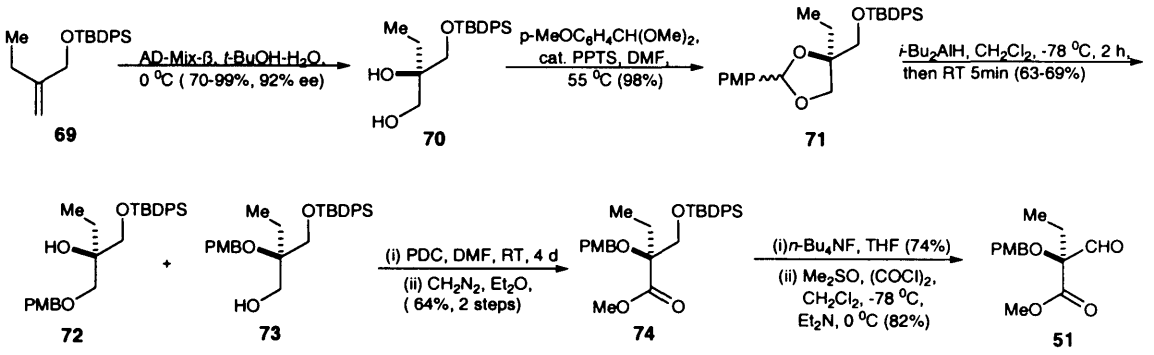
Intermediates **49** and **50** were successfully united by nucleophilic addition which gave a diastereomeric mixture of β-hydroxysulfones **64** (Scheme 14). These were directly subjected to Swern oxidation, and desulfonylation was accomplished via Smith's free-radical tri-*n*-butylstannane method[85] to afford ketone **65** as a single diastereoisomer. The next step was to install the (*E*)-tri-substituted alkene. Initially **65** was converted to the (*Z*)-enol triflate but efforts to perform a cross-coupling using Stille[86] or McMurry-Scott[87] protocols were unsuccessful. Consequently alkene **66** was obtained via Grignard addition and $POCl_3$-pyridine mediated dehydration. Unfortunately the dehydration was not very selective, it resulting in a 2.6:1 mixture of required **66** and the 1,1-disubstituted alkene. This mixture was selectively *O*-desilylated at the primary position, which facilitated chromatographic separation of the two isomers. Alcohol **67** was converted to phenylsulfone **68** in a further two steps.



Scheme 14 Synthesis of phenylsulfone **68**

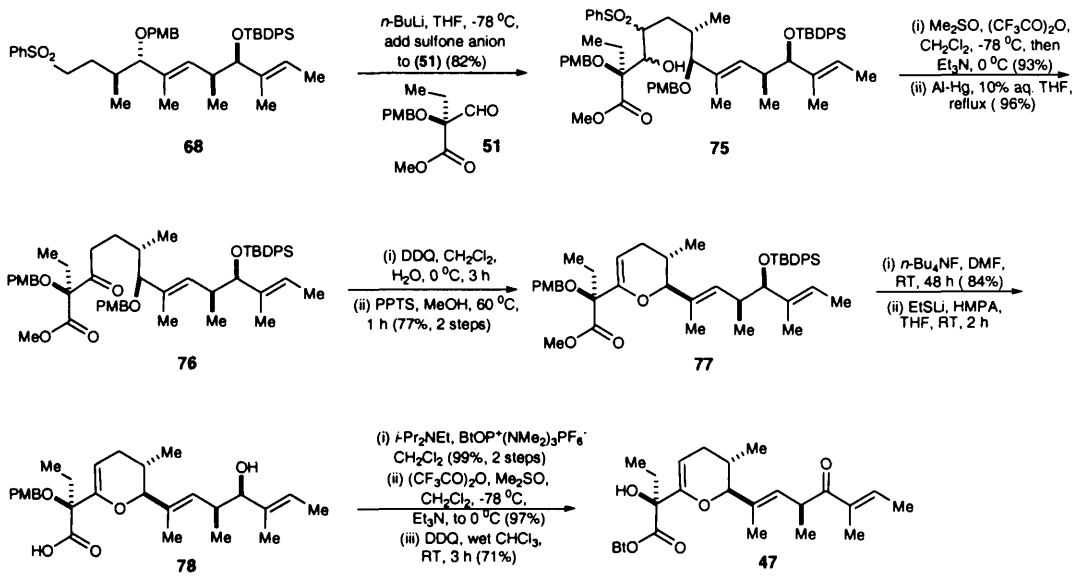
Synthesis of the chiral β-aldehydo ester **51** (Scheme 15) commenced with a Sharpless catalytic asymmetric dihydroxylation raction[88] on the 1, 1-disubstituted alkene **69** which delivered diol **70** in 99% yield and greater than 92% ee. As before the 1,2 diol was protected as a *p*-methoxybenzylidene acetal and reductive cleavage attempted with DIBAL. Unfortunately the regioselectivity of this reaction was not high, it affording a 2:1 mixture of **72** and **73** which,

fortunately, was readily separable by SiO_2 flash chromatography. A further four standard steps of oxidation, esterification, *O*-desilylation and Swern oxidation completed the synthesis of aldehyde **51.**



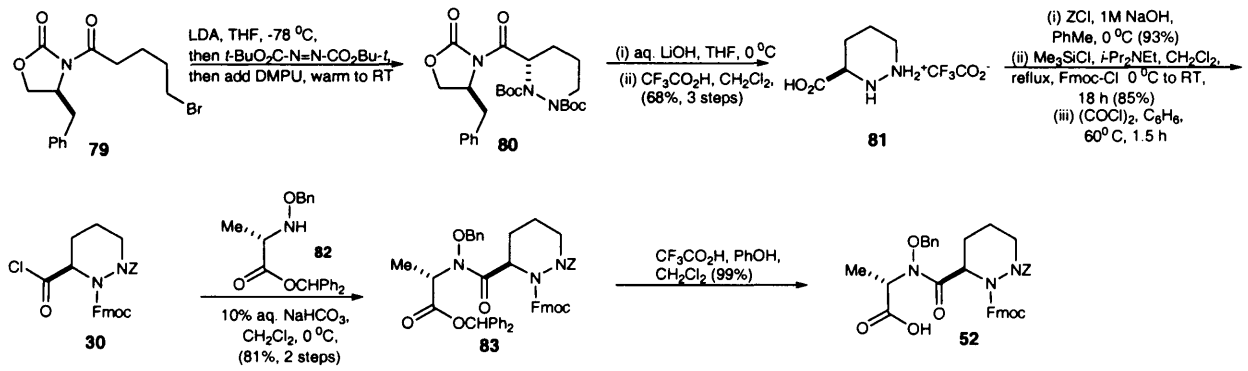
Scheme 15 Synthesis of aldehyde **51**

Intermediates **51** and **68** were coupled together by adding the sulfone anion of **68** to aldehyde **51**. Oxidation and Corey-Chaykovsky desulfonylation[89] using aluminium mercury amalgam then gave ketone **76** (Scheme 16). A chemoselective removal of the more electron rich, and less sterically hindered, secondary PMB ether in **76** was then attempted; this provided a mixture of linear δ-hydroxy ketone and the two α- and β-ring-closed hemiketals. In order to obtain a single compound this mixture was subjected to dehydration/cyclisation with methanol and PPTS at 60 °C to obtain glycal **77**. The methyl ester of **77** was now cleaved to obtain acid **78** in two steps. Construction of the activated ester **47** was thereafter accomplished by treatment with Castro's BOP reagent, Swern oxidation, and deprotection of the tertiary PMB ether with DDQ[90] in wet chloroform.



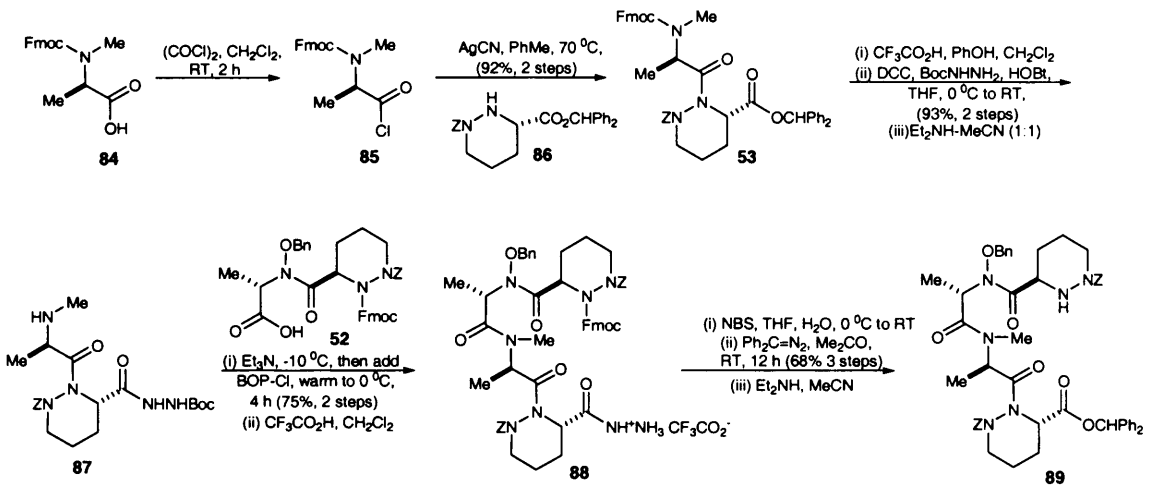
Scheme 16 Synthesis of the A83586C pyran **47**

A key requirement for the synthesis of the A83586C cyclodepsipeptide ring was the development of new methodology for accessing both enantiomers of piperazic acid. For this a tandem electrophilic hydrazination-nucleophilic cyclisation protocol[91] was devised (Scheme 17). Thereafter utilizing 3-(*R*)-*N*(1)-*Z*-*N*(2)-Fmoc-piperazic acid chloride **30** and *N*-benzyloxy-L-alanine diphenylmethyl ester **82**, dipeptide **83** was prepared (Scheme 17).



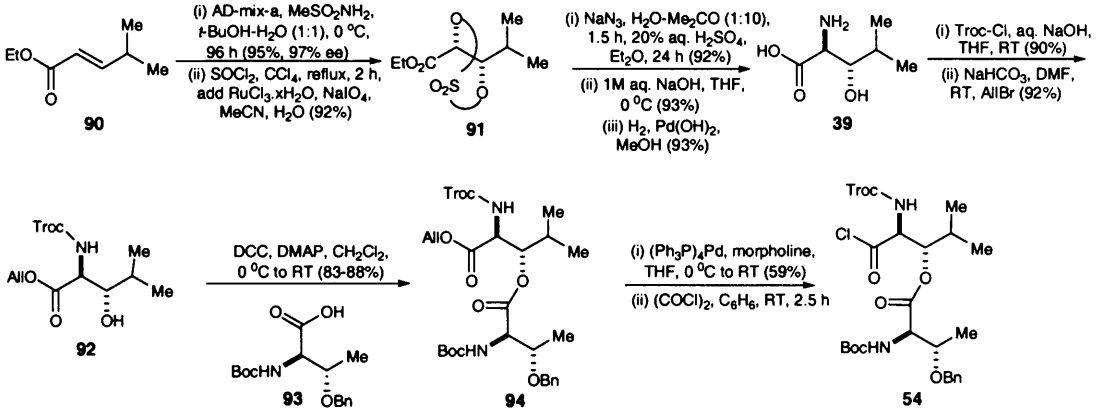
Scheme 17 Synthesis of A83586C depsipeptide **52**

Dipeptide **53** (Scheme 18) was prepared using a AgCN-assisted coupling between Fmoc-*N*(Me)-D-alanyl chloride **85** and 3-(*S*)-piperazic acid derivative **86**. Because removal of the Fmoc protecting group from **53** caused formation of the diketopiperazine, the diphenylmethyl ester in **53** was converted to a *t*-butylcarbazate[92] to give **87**. This now permitted deprotection of the Fmoc group and the subsequent coupling of **87** to dipeptide **52** using BOP-Cl for acid activation. After excising the Boc group with TFA and oxidation of the acylhydrazine with NBS[93] in THF/H_2O, the ensuing acid was esterified with diphenyldiazomethane and the Fmoc group cleaved to obtain tetrapeptide **89**.



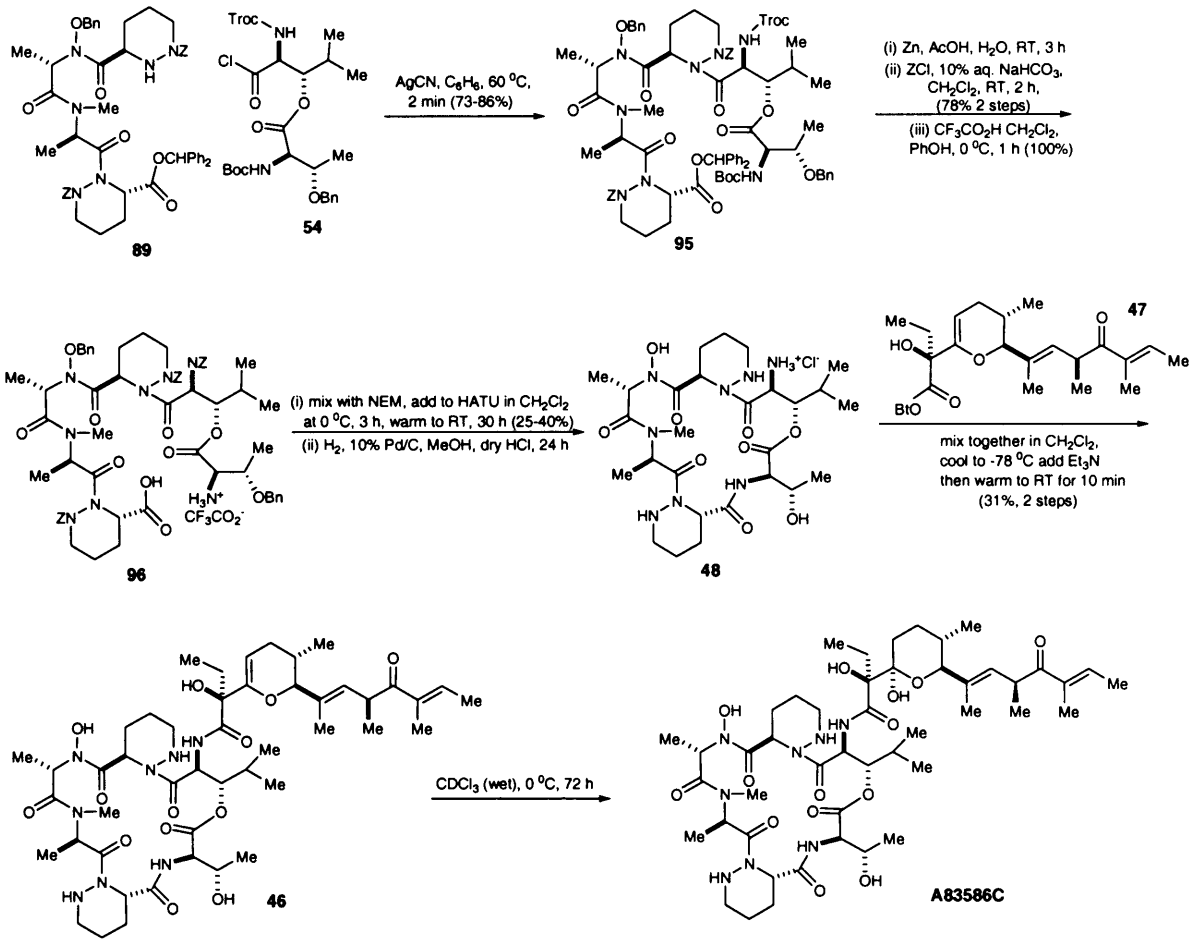
Scheme 18 Synthesis of A83586C tetrapeptide **89**

The depsipeptide fragment contains a (2*S*, 3*S*)-3-hydroxyleucine residue and again the Hale[94] group developed a new process for securing this compound (Scheme 19) which relied upon Sharpless AD and cyclic sulfate chemistry to install the *anti*-aminoalcohol motif in (2*S*, 3*S*)-3-hydroxyleucine **39**. Further functional group protection resulted in alcohol **92**, which coupled efficiently to **93** through a standard DMAP-assisted DCC coupling. *O*-Deallylation of **94** with Pd(0) and morpholine, and chlorination provided the didepsipeptide **54**.



Scheme 19 Synthesis of A83586C ester **54**

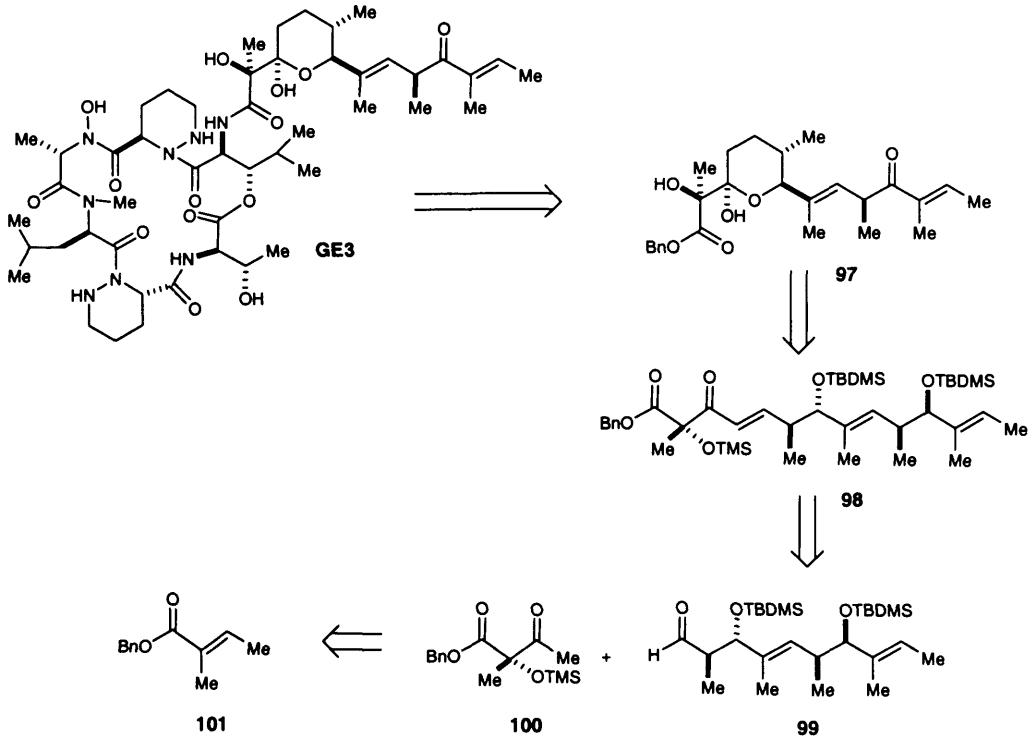
The AgCN mediated amidation between tetrapeptide **89** and depsipeptide **54** afforded the linear hexadepsipeptide **95** in 73-86% yield (Scheme 20). Z for Troc group interchange and acidolysis of the terminal Boc and diphenylmethyl ester groups with TFA and phenol thereafter provided the macrolactamisation precursor **96**. Ring closure with Carpino's HATU[95] reagent, under high dilution conditions, delivered the desired macrolactam in 25-40% yield. Final deprotection by catalytic hydrogenation over a Pd-C catalyst in methanolic HCl provided the hydrochloride salt of the A83586C cyclodepsipeptide ring **48**. One equivalent of methanolic HCl was added to the reaction vessel to protonate the hydroxyleucine amine as soon as it was liberated to prevent *O*- to *N*-acyl migration.[96] With both key fragments in hand, the asymmetric total synthesis of A83586C was accomplished by a chemo- and regio-selective coupling between **47** and **48** mediated by Et_3N, which provided the glycal **46**. The final step of the synthesis was the regioselective hydration of **46** in wet $CDCl_3$ to obtain A83586C in 31% overall yield for the last two steps.



Scheme 20 Completion of the total synthesis of A83586C

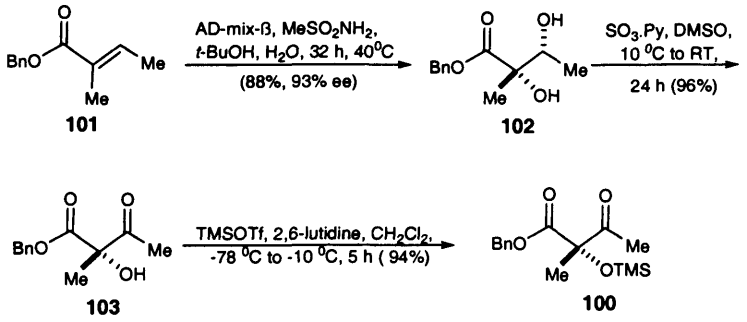
3.3 Synthesis of the GE3 Acyl Side Chain

In 2002 the Hamada group reported the first stereoselective synthesis[97] of the acyl side chain segment of GE3; their retrosynthetic plan is depicted in Scheme 21. Hemiacetal **97** was thought accessible from intermediate **98**, which itself would be constructed via an aldol condensation between **99** and **100**. The quaternary centre of **100** would be installed by a Sharpless' asymmetric dihydroxylation on tiglic ester **101**. Fragment **99** would itself be synthesised by a diastereoselective *syn* aldol condensation with the chiral auxilliary developed by Evans followed by a Paterson diastereoselective *anti* aldol condensation.

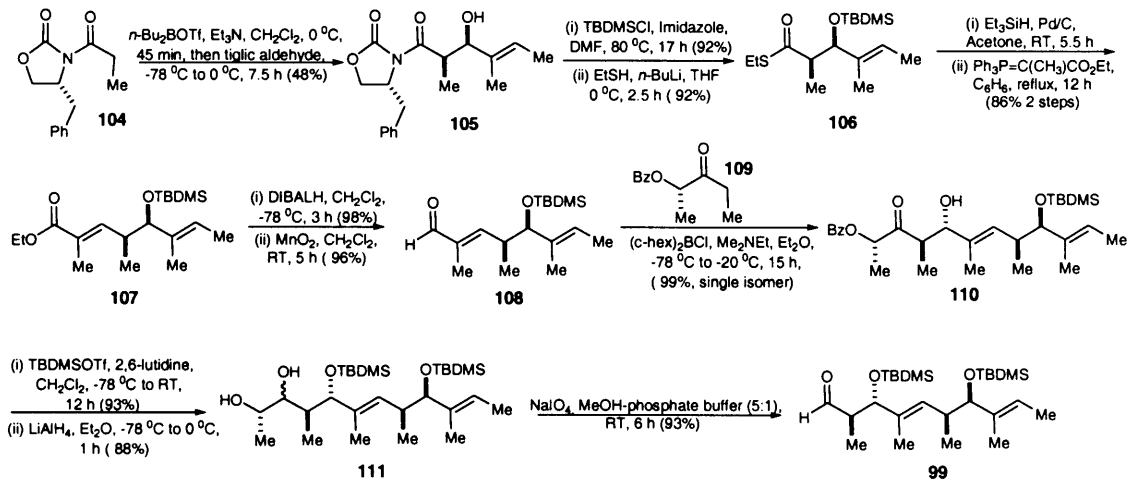


Scheme 21 Hamada's retrosynthesis of the GE3 pyran **97**

Intermediate **100** was formed as illustrated (Scheme 22) in three steps from benzyl tiglate **101**, the key quaternary asymmetric centre being installed via an enantiocontrolled Sharpless dihydroxylation with AD-mix-β, which proceeded in 88% yield and 93% ee.



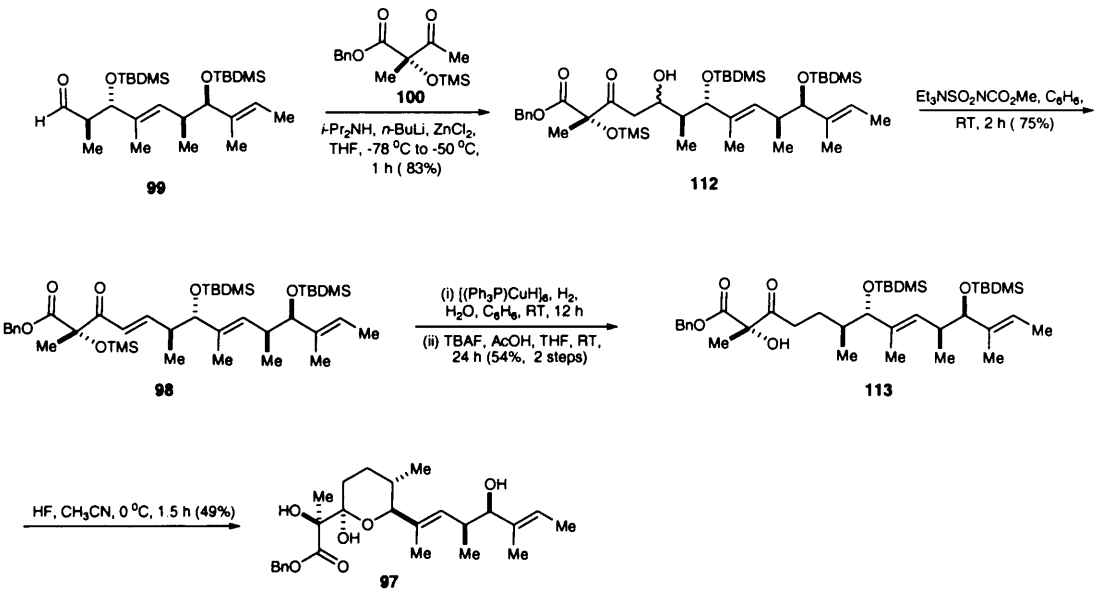
Scheme 22 Synthesis of diketoester **100**



Scheme 23 Synthesis of aldehyde **99**

Construction of fragment **99** is depicted in Scheme 23. The four stereocenters of **99** were set by aldol reactions. The first involved an Evans *syn*-aldol reaction between propionamide **104** and tiglic aldehyde. The second was a Paterson *anti*-aldol reaction between the dicyclohexyl borinate of ketone **109** and aldehyde **108**, which eventually yielded ketone **110** as a single isomer.

With the two key intermediates **99** and **100** in hand the synthesis of the target hemiacetal **97** was completed in 5 steps (Scheme 24). Coupling of the two fragments was achieved by an aldol condensation with LDA-$ZnCl_2$ in THF. A 4:1 mixture of aldol adducts **112** was formed in 83% yield. A dehydration, olefin saturation and TMS ether cleavage produced alcohol **113**. Hemiacetal formation concurrent with removal of the two TBDMS ether protecting groups completed the synthesis of the acyl side chain segment **97**.



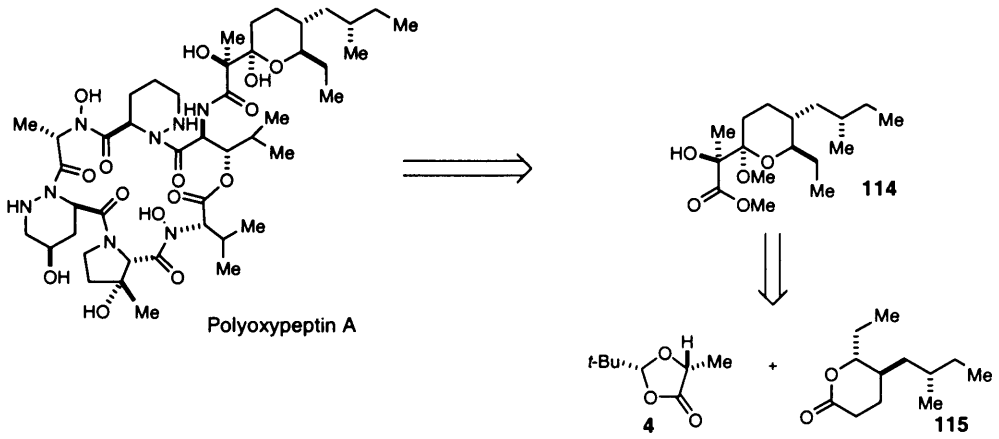
Scheme 24 Total synthesis of GE3 pyran **97**

3.4 Synthetic studies on Polyoxypeptin A

So far, no asymmetric total synthesis of the potent apoptosis inducer polyoxypeptin A has been achieved, despite considerable progress by a number of research groups. Synthesis of the acyl side chain of this molecule has been accomplished by the groups of Kobayashi[98], Kurosu[99] and Yao.[100] The cyclodepsipeptide core of polyoxypeptin A contains two amino acids that are not present in any of the other A83586C/GE3 family members. These are the novel (2*S*, 3*R*)-3-hydroxy-3-methylproline and (3*R*, 5*R*)-5-hydroxypiperazic acid residues. (2*S*, 3*R*)-3-Hydroxy-3-methylproline was first stereoselectively synthesised by Kobayashi[101] and further syntheses were later reported by Yao[102] and Hamada.[103] An efficient enantiospecific total synthesis of (3*R*, 5*R*)-5-hydroxypiperazic acid was achieved by Hale[104] and this was followed by a synthesis by Danishefsky.[105]

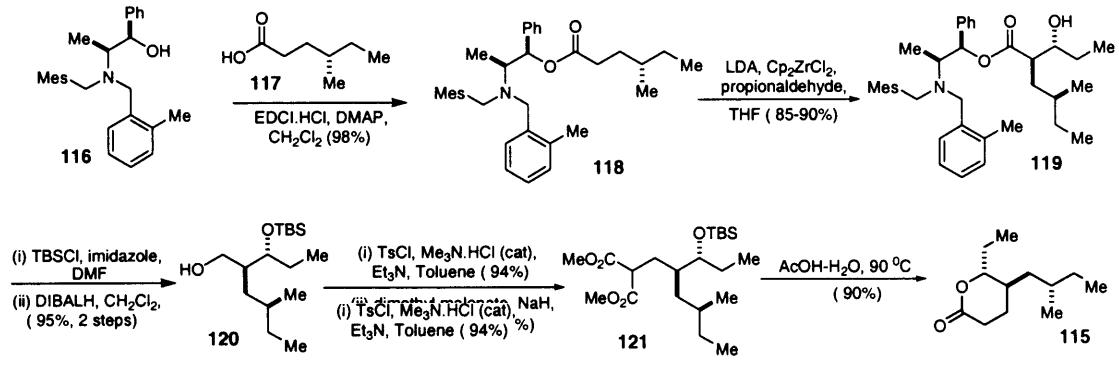
3.4.1 Synthesis of the polyoxypeptin A acyl side chain

The acyl side chain of polyoxypeptin A **114** is almost identical to that of L-156,602, the difference being the alkyl substituent on the tetrahydropyran ring (ethyl in polyoxypeptin A and methyl in L-156,602). The groups of Yao and Kurosu believed they could access acyl side chain **114** via the reaction between lactone **115** and Seebach's ester **4** (Scheme 25), the method used effectively by Caldwell in the synthesis of L-156,602, described earlier (Scheme 4).



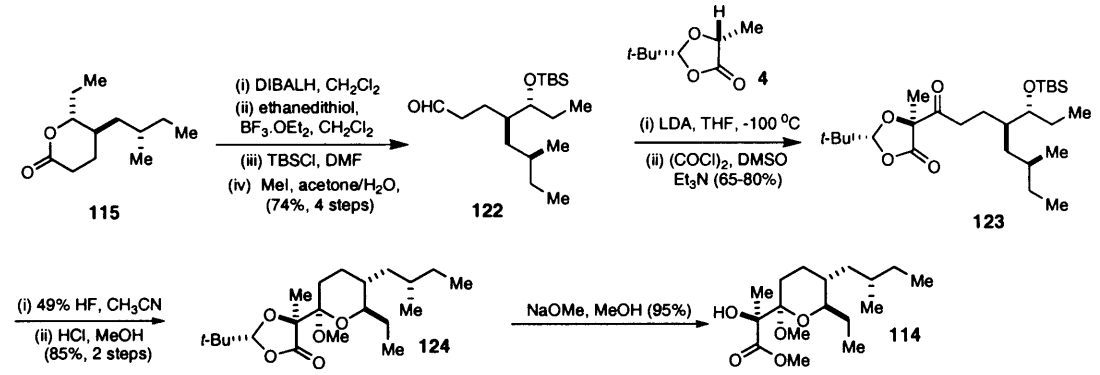
Scheme 25 Yao and Kuroso's retrosynthesis of Polyoxypeptin A pyran **114**

The route used by Kurosu to access lactone **115** is depicted in Scheme 26. The key step involved a highly diastereoselective *anti*-aldol reaction. The *N*, *N*-dibenzyl norephedrine derivative **116** was esterified with known chiral acid **117** to give aldol precursor **118**. Conversion of **118** to the *E*-enolate using LDA and Cp_2ZrCl_2, followed by aldolization with propionaldehyde afforded the *anti*-aldol product **119** in 85-90% yield with 98% *de*. Protection of the ensuing alcohol as a TBS ether, followed by reductive cleavage of the chiral auxiliary furnished alcohol **120**. The primary alcohol was converted to its tosylate which was reacted with the cesium methyl malonate anion to give cyclisation precursor **121**. Treatment of malonate adduct **121** with 80% AcOH at 90^{0}C afforded required lactone **115**.



Scheme 26 Kuroso's route to lactone **115**

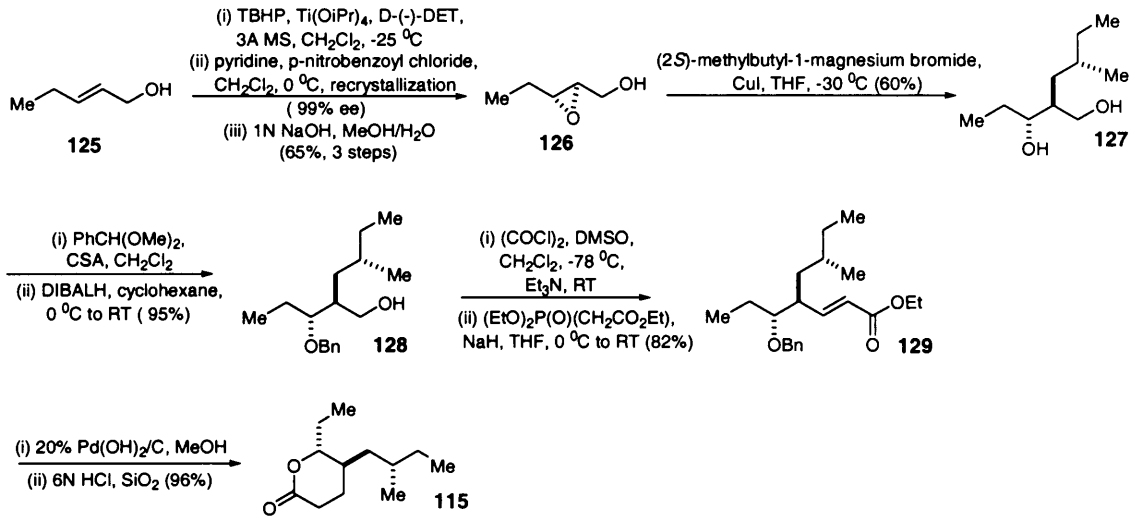
Despite the attempts of the Kurosu group, the proposed coupling reaction between lactone **115** and Seebach ester **4** was unsuccessful. In order to facilitate this reaction the lactone **115** was converted in four steps to the corresponding acyclic aldehyde **122** (Scheme 27). This time the lithium enolate derived from **4** coupled productively to **122**, and following Swern oxidation gave β-keto ester **123**. The final acyl side chain target **114** was acquired in a further three steps.



Scheme 27 Kuroso's synthesis of Polyoxypeptin A pyran **114**

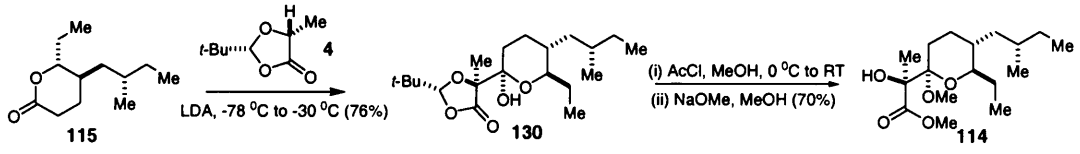
Yao and coworkers also made lactone **115** a key subtarget in their synthetic approach to polyoxypeptin A. The short and efficient route employed by Yao to access the required lactone **115** is depicted in Scheme 28. The highlights of the synthesis involved a Sharpless asymmetric epoxidation of alcohol **125**, followed by a regioselective epoxide opening by the cuprate reagent

derived from (*S*)-1-bromo-2-methylbutane to give 1,3-diol **127**. A further six steps furnished the requisite lactone **115**.



Scheme 28 Yao's route to lactone **115**

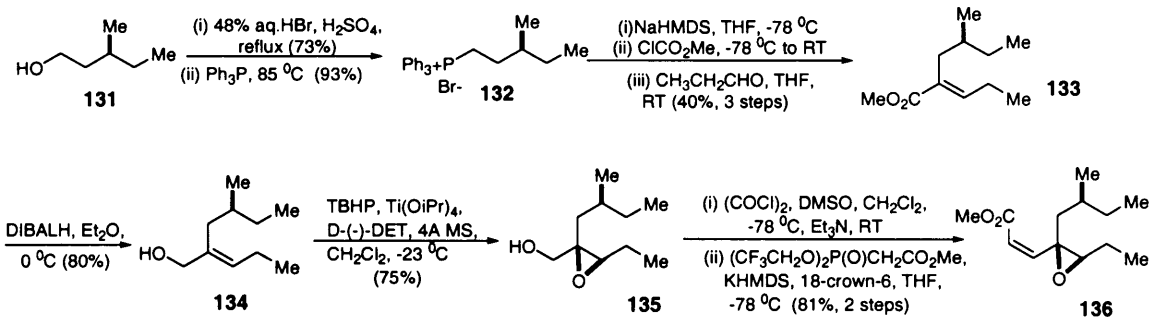
With lactone **115** in hand, Yao conducted the coupling reaction with Seebach ester **4** and, unlike the failed attempts by Kurosu at this stage, compound **130** was obtained as a single diastereoisomer in 76% yield (Scheme 29). Yao found that for maximum yields two equivalents of the lithium enolate of **4** were required, and that the temperature of the reaction had to be increased from -78 °C to -30 °C. Treatment of **130** with methanolic HCl afforded the intermediate methyl pyranoside which was subsequently transesterified with NaOMe to give target ester **114**.



Scheme 29 Yao's synthesis of Polyoxypeptin A pyran **114**

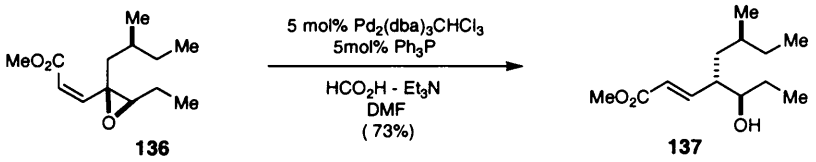
A route to the acyl side chain of polyoxypeptin A that did not involve the use of Seebach ester **4** was engineered by Kobayashi (Schemes 30, 31, 32). The key feature of their synthesis

was the stereospecific palladium-catalyzed hydrogenolysis of (*Z*)-alkenyloxirane **136** to *anti*-hydroxyalkenoate **137** (Scheme 31).



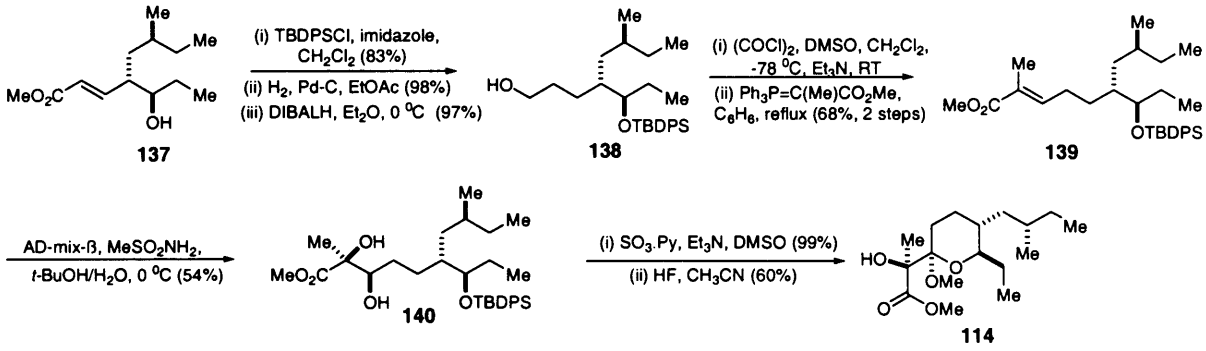
Scheme 30 Kobayashi's synthesis of (*Z*)-alkenyloxirane **136**

The epoxyalkenoate precursor **136** for the Pd-catalyzed hydrogenolysis was synthesized in nine steps from the chiral alcohol **131**, itself readily prepared from L-(+)-isoleucine (Scheme 30). (*Z*)-Alkenyloxirane **136** was treated with 5 mol% $Pd_2(dba)_3.CHCl_3$, 5 mol% Ph_3P, HCO_2H, and Et_3N in DMF at room temperature to afford desired *anti*-isomer **137** in 73% yield and high stereospecificity (*anti:syn* = 96:4) (Scheme 31).



Scheme 31 Kobayashi's Pd-catalyzed hydrogenolysis of (*Z*)-alkenyloxirane **137**

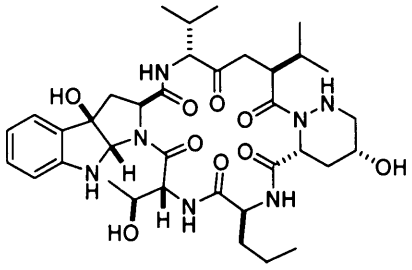
Completion of the acyl side chain fragment is shown in Scheme 32. Alcohol **137** was converted to its TBDPS ether, whose α,β-unsaturated ester moiety was completely reduced in two steps to give alcohol **138**. Swern oxidation and Wittig homologation provided the tri-substituted (*E*)-alkene **139** which was subjected to Sharpless asymmetric dihydroxylation to produce diol **140** as a single isomer. Oxidation of the secondary hydroxyl group and deprotection of the silyl group with concomitant hemiketal formation furnished the target fragment **114**.



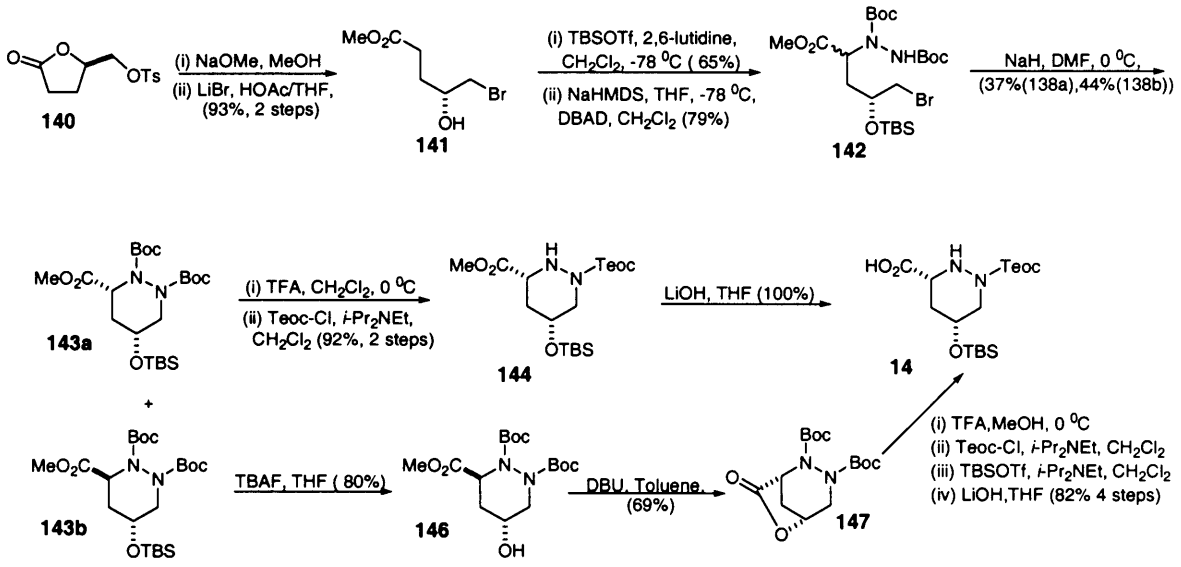
Scheme 32 Kobayashi's synthesis of Polyoxypeptin A pyran **114**

3.4.2 Synthesis of (3*R*, 5*R*)-5-Hydroxypiperazic acid

Danishefsky's synthesis of a protected version of (3*R*, 5*R*)-5-hydroxypiperazic acid is illustrated in Scheme 34. This synthesis was not devised for the polyoxypeptin A synthetic problem but for work on the total synthesis of himastatin (Scheme 33); an antitumour cyclodepsipeptide that also contains this residue. The starting point was butyrolactone **140**, derived from D-glutamic acid, which was converted to the epoxy ester with NaOMe and subsequently opened with LiBr in HOAc to give hydroxy ester **141** (Scheme 34). Protection of the hydroxyl as a TBS ether and amination with DBAD gave approximately a 1:1 diastereomeric mixture of **142**, which after cyclisation afforded piperazic esters **143a** and **143b**. Desired piperazic ester **143a** was isolated, the Boc groups removed and the more reactive *N*(1) nitrogen protected with a TEOC group to give **144**. Conversion to amino acid **145** was through hydrolysis with LiOH. The undesired piperazic ester **143b** could still be converted to desired compound **14** in six steps as shown.



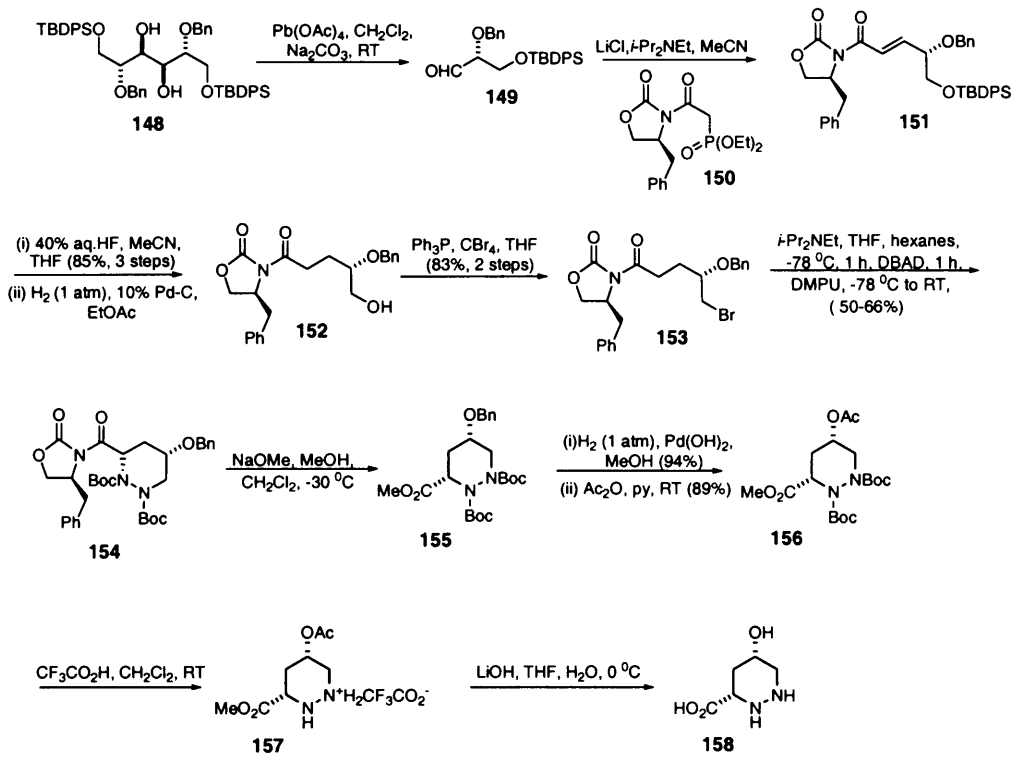
Scheme 33 Himastatin



Scheme 34 Danishefsky's synthesis of a protected version of (3*R*, 5*R*)-5-hydroxypiperazic acid

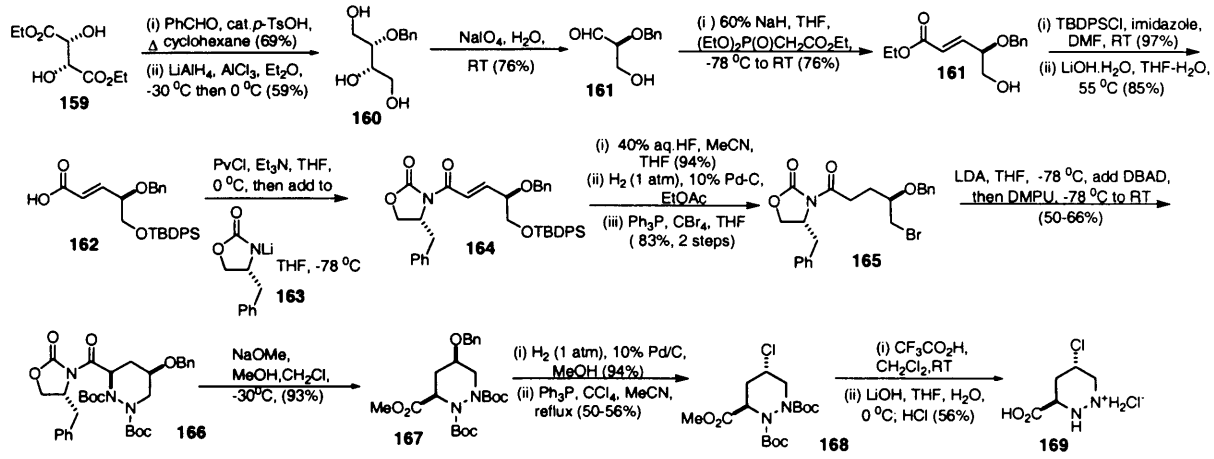
The Hale group enantiospecific synthesis of (3*S*, 5*S*)-5-hydroxypiperazic acid (Scheme 35) commences from the known D-mannitol derivative **148**. Diol **148** was oxidatively cleaved with $Pb(OAc)_4$ and the ensuing aldehyde **149** was olefinated under Wittig-Horner conditions with known phosphonate[106] **150** to furnish **151** as a single geometrical isomer. The silyl group was removed, the olefin reduced via hydrogenation with 10% Pd-C, and the alcohol converted to bromide **153**. This was subjected to a tandem electrophilic hydrazination/nucleophilic cyclisation reaction to yield **154** in 50-66% yield after chromatographic purification and crystallisation.

The chiral auxiliary was cleaved with NaOMe in MeOH to give **155**. The final (3*S*, 5*S*)-5-hydroxypiperazic acid **158** was obtained by debenzylation and *O*-acetylation to furnish **156**. The Boc group cleavage with CF_3CO_2H in CH_2Cl_2 afforded salt **157** which when treated with excess LiOH in THF/H_2O gave **158**.



Scheme 35 Hale's enantiospecific synthesis of (3*S*, 5*S*)-5-hydroxypiperazic acid

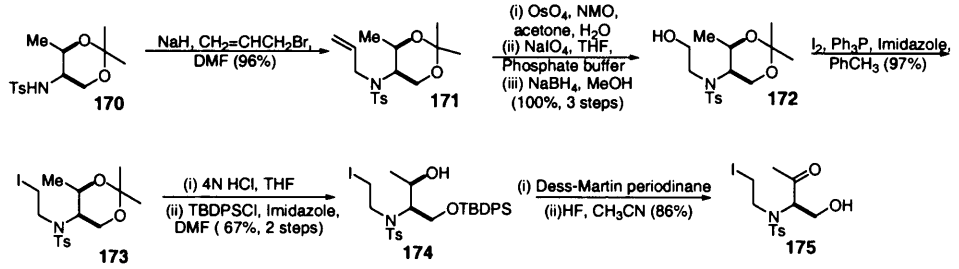
To prepare the (3*R*, 5*R*)-enantiomer of **158** a new synthetic approach was developed which also allowed the formation of (3*R*,5*S*)-5-chloropiperazic acid. The starting point was commercially available diethyl-L-tartrate **159** (Scheme 36) which was converted to the benzylidene acetal and then reduced with AlH_3 to obtain the polyol **160**. Oxidation of **160** followed by Wittig homologation provided the known 1, 2-disubstituted (*E*)-alkene **161**. Protection of the hydroxyl as a silyl ether and hydrolysis of the ester afforded the α, β-unsaturated acid **162**. This was converted to the mixed pivalic acid anhydride and this added crude to the lithiated chiral auxiliary **163** to provide **164**. The sequence of steps to the final (3*R*, 5*S*)-5-chloropiperazic acid **169** paralleled those described previously in the synthesis of (3*S*, 5*S*)-5-hydroxypiperazic acid.



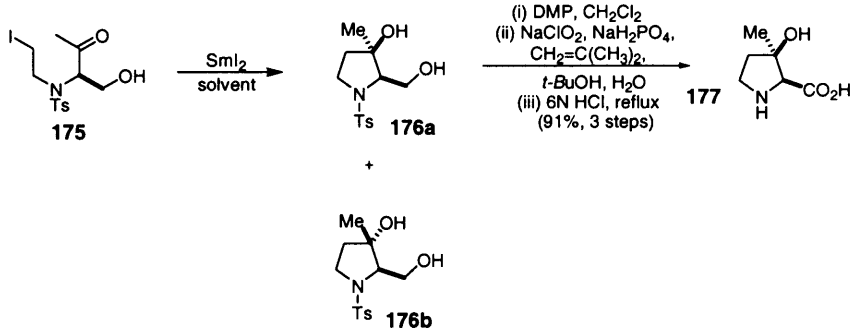
Scheme 36 Hale's enantiospecific synthesis of (3*R*, 5*S*)-5-chloropiperazic acid

3.4.3 Synthesis of (2*S*, 3*R*)-3-Hydroxy-3-Methylproline

Hamada's stereoselective synthesis of (2*S*,3*R*)-3-hydroxy-3-methylproline **177** (Schemes 37 and 38) was achieved with the use of a SmI_2-mediated diastereoselective cyclization. The precursor for this cyclization was iodoketone **175** synthesized as shown in Scheme 37 from (2*S*, 3*R*)-threonine. After much experimentation it was found that iodoketone **175** exclusively afforded the cyclised product **176a** in 75% yield and ratio of 97:3, when treated with SmI_2 in THF-HMPA (10:1) at -78 ^{0}C to -55 ^{0}C for 1.5 h (Scheme 38). Interestingly under similar cyclization conditions, the protected hydroxyl version of **175** gave a mixture of diastereoisomers, suggesting a role for the free hydroxyl as a chelator with the SmI_2.



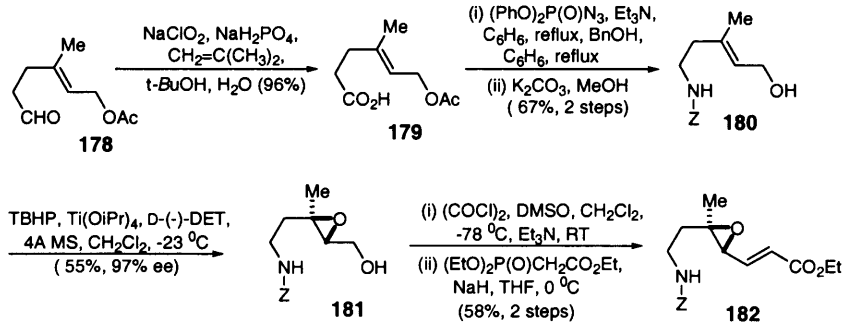
Scheme 37 Hamada's synthesis of iodoketone **175**



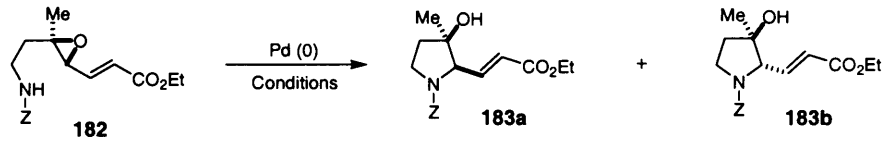
Scheme 38 Hamada's stereoselective synthesis of (2*S*, 3*R*)-3-hydroxy-3-methylproline

The synthesis was completed in a further three steps. Two step oxidation of the primary alcohol **176a** to the acid and removal of the *N*-tosyl group with HCl afforded **177** in 91% for the three steps.

Kobayashi's synthesis of (2*S*, 3*R*)-3-hydroxy-3-methylproline **177** was accomplished with the use of a palladium-catalysed intramolecular *N*-allylation of an alkenyloxirane (Scheme 40). The precursor alkenyloxirane **182** was synthesised from geraniol derivative **178** as depicted in Scheme 39. The optimised conditions for the Pd-catalyzed cyclisation were refluxing **182** in THF for 5h in the presence of 5 mol% $Pd(PPh_3)_4$ (Scheme 40). This afforded a 9:1 ratio of pyrrolidine derivatives **183a** and **183b**. Longer reaction times were highly detrimental, with no product being observed; the presence of base led to a complex mixture of products.

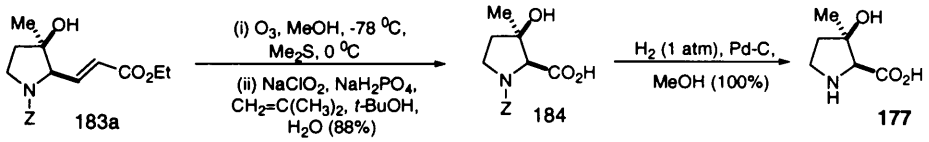


Scheme 39 Kobayashi's synthesis of alkenyloxirane **182**



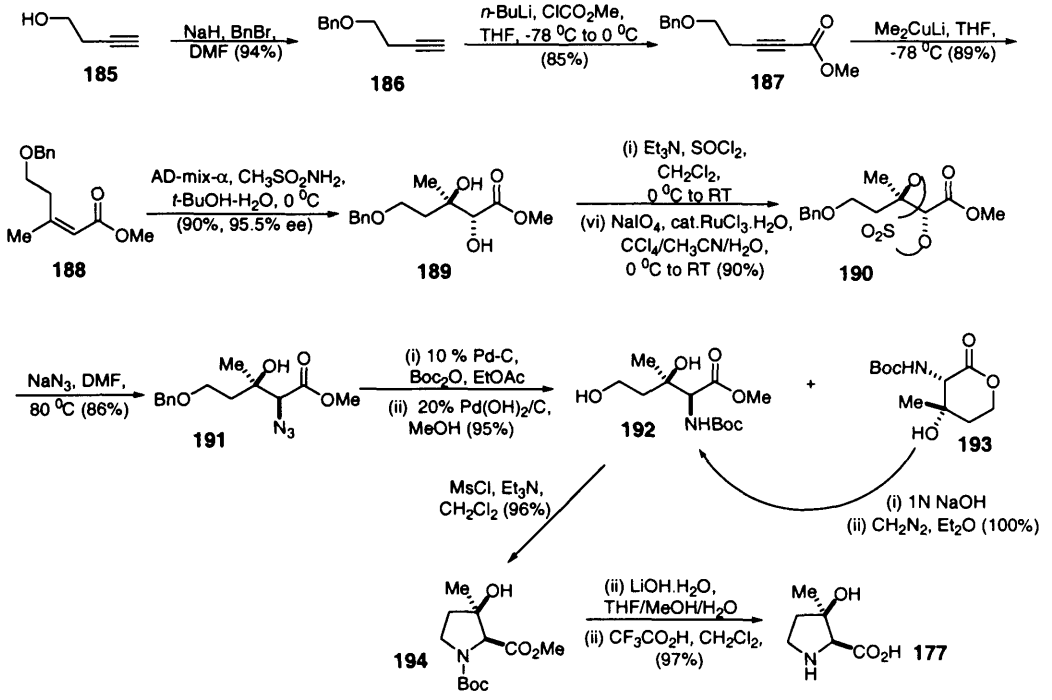
Scheme 40 Kobayashi's Pd-catalyzed cyclisation of alkenyloxirane **182**

To complete the synthesis (Scheme 41), the olefin of pyrrolidine derivative **183a** was oxidatively cleaved with O_3, and the aldehyde oxidised further with $NaClO_2$ to give acid **184**. A final Pd-C catalysed hydrogenation of the benzyloxycarbonyl group furnished **177**.



Scheme 41 Kobayashi's stereoselective synthesis of (2*S*, 3*R*)-3-hydroxy-3-methylproline

Yao's synthesis of (2*S*,3*R*)-3-hydroxy-3-methylproline invoked a Sharpless asymmetric dihydroxylation followed by regioselective opening of cyclic sulphate **190** by sodium azide (Scheme 42). 3-Butyn-1-ol **185** was protected as a benzyl ether. Treatment of butyne ether **186** with *n*-BuLi and trapping of the resultant lithium acetylide with methyl chloroformate gave **187**. Addition of **187** to $(CH_3)_2CuLi$ in THF at -78^{0}C afforded the trisubstituted olefin **188**. This was subjected to a Sharpless asymmetric dihydroxylation to yield diol **189** in 90% yield and 95.5% ee. The diol was converted to the cyclic sulphate **190**, which was regioselectively opened with NaN_3 to give the α-azido-β-hydroxy ester **191**. Hydrogenolysis of the azido group in the presence of catalytic Pd-C and Boc_2O followed by removal of the *O*-benzyl group with catalytic $Pd(OH)_2$-C furnished diol **192** in 72% yield alongside 23% of the by-product lactone **193**. Lactone **193** was, however, convertible back to the desired diol **192** by alkaline hydrolysis and esterification.



Scheme 42 Yao's stereoselective synthesis of (2*S*, 3*R*)-3-hydroxy-3-methylproline

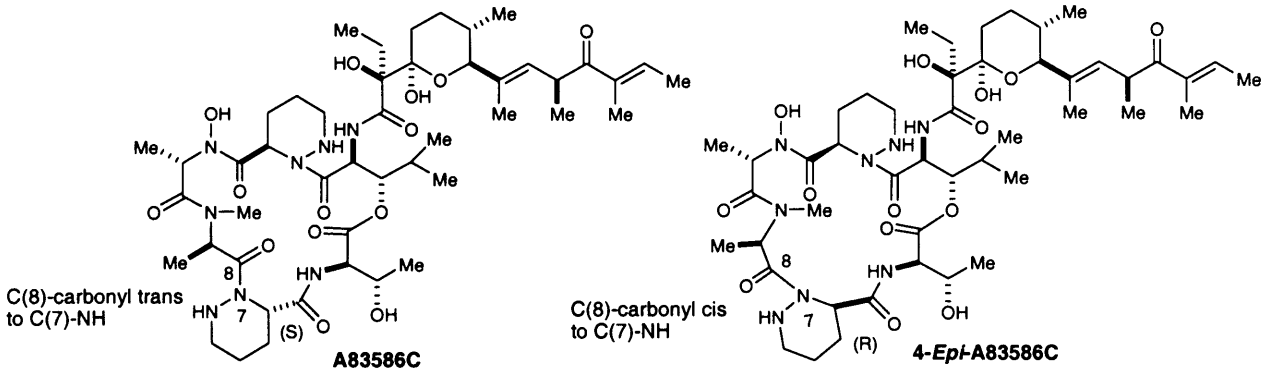
A three step sequence afforded the final product. Treatment of diol **192** with MsCl and Et_3N in CH_2Cl_2 gave the desired pyrrolidine **194** in 91% yield. The methyl ester was hydrolysed with LiOH and the Boc group removed with CF_3CO_2H to access the final product **177**.

CHAPTER 4 RESULTS AND DISCUSSION

Molecules of the A83586C/GE3 class have been suggested to function as antitumour agents by selectively preventing deregulated E2F-DP transcription factors from binding to and activating target genes encoding for proteins involved in cell growth and proliferation. Confirming whether they do this or whether they operate by another mechanism is currently a topic of great interest, as it could potentially lead to the detailed characterization and validation of important new drug targets for the possible control of proliferative diseases such as cancer and atherosclerosis. With regard to the E2F inhibitory mechanism, several modes of action are currently being considered. One hypothesis is that members of the A83586C/GE3 family are binding to individual E2F and DP proteins and preventing them from heterodimerizing. The E2F-DP subunit is considered essential for high-affinity binding to target DNA, and this in turn is a prerequisite for gene transcription. Alternatively, molecules of this class might be complexing to individual E2F-DP heterodimers and sterically blocking their interaction with various target genes. E2F-DP drug binding could also be halting the recruitment of various transcriptional repressors such as HDAC1 (class 1 histone deacetylase) and CHRBP (cell cycle genes homology region protein) and thus promoting the transcription of genes that would otherwise be repressed. In light of the controversy surrounding their mode of antitumour activity, our group initiated a total synthesis programme on the A83586C class, for the purpose of preparing novel analogues that could probe these mechanistic issues further, and with a view to identifying several new, structurally less elaborate drug candidates with enhanced antitumour effects and improved toxicological properties.

As already discussed, our group completed the first asymmetric total synthesis of A83586C back in 1997. During the original synthetic work on A83586C, our group also completed a synthesis of its 4-*epi*-analogue.[115] 4-*Epi*-A83586C has a (3*R*)-piperazic acid component replacing the (3*S*)-piperazic acid residue of the natural product (Scheme 43). While the latter modification does actually serve to markedly improve the overall yield of macrolactamisation from 25-40% to 70%, it has a detrimental effect on antitumour potency; the 4-*epi*-analogue being much less potent

as an antitumour drug than the parent A83586C. High-field NMR studies of the solution conformation of 4-*epi*-A83586C have shown that the C(8)-carbonyl adopts a *cis*-orientation relative to the C(7)-piperazine-NH, which is opposite to A83586C where a *trans*-relationship is believed to exist between these two groupings. This suggests that significant conformational perturbations to the "Southern Hemisphere" of these natural products have an adverse effect on antitumour activity.

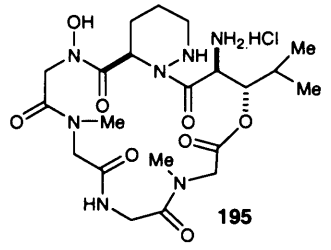


Scheme 43 Orientation of C-(8) carbonyl in A83586C and 4-*Epi*-A83586C

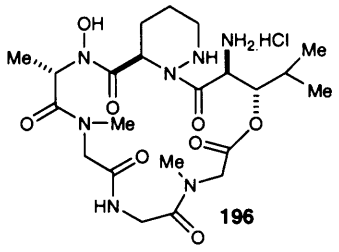
Additional biological testing on the A83586C cyclodepsipeptide hydrochloride, lacking the acyl side-chain, revealed that it was totally devoid of all antitumour activity. These early SAR studies have highlighted the importance of having a lipophilic acyl side-chain connected to the hydroxyleucine nitrogen if one wishes to observe good antitumour effects. In light of the above observations we decided to synthesise a range of different A83586C/GE3 analogues that would:

- Possess conformationally-flexible cyclodepsipeptide rings that would aid us in understanding whether structural rigidity and complexity in the "Southern Hemisphere" is a requirement for good antitumour activity;
- Have more rigid cyclodepsipeptide skeleta with conformational properties more closely approximating that of A83586C; and
- Have considerably simplified pyran acyl side-chains with enough hydrogen bonding and hydrophobic functionality to give them a realistic chance of interacting strongly with protein or gene targets.

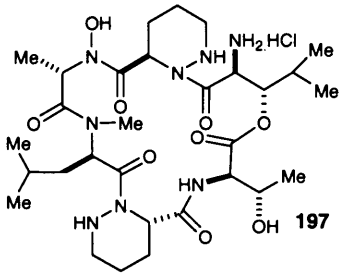
The cyclodepsipeptide rings that would serve as core scaffolds for the construction of these analogue structures are shown in Scheme 44. The verucopeptin cyclodepsipeptide **195** and the A83586C/verucopeptin cyclodepsipeptide **196** were chosen due to them possessing a rigid but flexible structure when compared to A83586C, and also because they were considerably simplified compared to the A83586C and GE3 cyclodepsipeptides and hence easier to synthesise. Moreover verucopeptin displayed good antitumour potency.



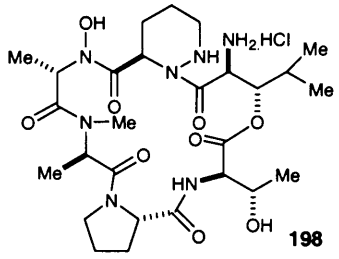
The Verucopeptin Cyclodepsipeptide



An A83586C/Verucopeptin Hybrid Cyclodepsipeptide



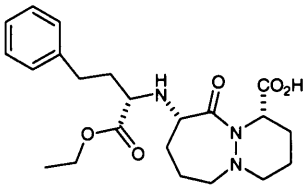
The GE3 Cyclodepsipeptide



An L-Proline Modified Mimetic of the A83586C Cyclodepsipeptide

Scheme 44 Target cyclodepsipeptide rings

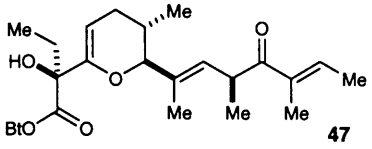
We also selected the L-proline-modified cyclodepsipeptide **198** as a core scaffold candidate, as we felt that this would adopt a conformation similar to A83586C and GE3. Our decision to replace the (3*S*)-piperazic acid residue with an L-Pro was based upon the fact that (3*S*)-piperazic acid had previously functioned as a very effective mimic of L-proline in ACE-inhibitory drugs such as cilazapril (Scheme 45).[116]



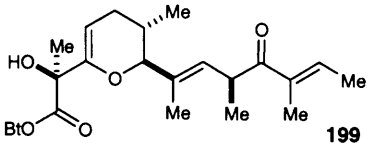
Scheme 45 Cilazapril

The GE3 cyclodepsipeptide was chosen due to GE3 being the most potent antitumour agent of this class so far discovered. Moreover we wished not only to utilize it for our analogue programme but to also attempt its asymmetric total synthesis.

The route to the A83586C acyl side-chain had already been established within our group (Chapter 3) and so during the course of my PhD studies, I employed this previously developed route for all the analogue work that was carried out. Significantly though, I was able to scale-up the route to compound **47** such that multi-gram quantities were finally produced. I also attempted to complete an asymmetric synthesis of the corresponding GE3 acyl side-chain **199** with a view to achieving the first asymmetric total synthesis of GE3 itself, and using it for coupling studies with all the other cyclodepsipeptides that would be prepared.



The A83586C acyl side chain



The GE3 acyl side chain

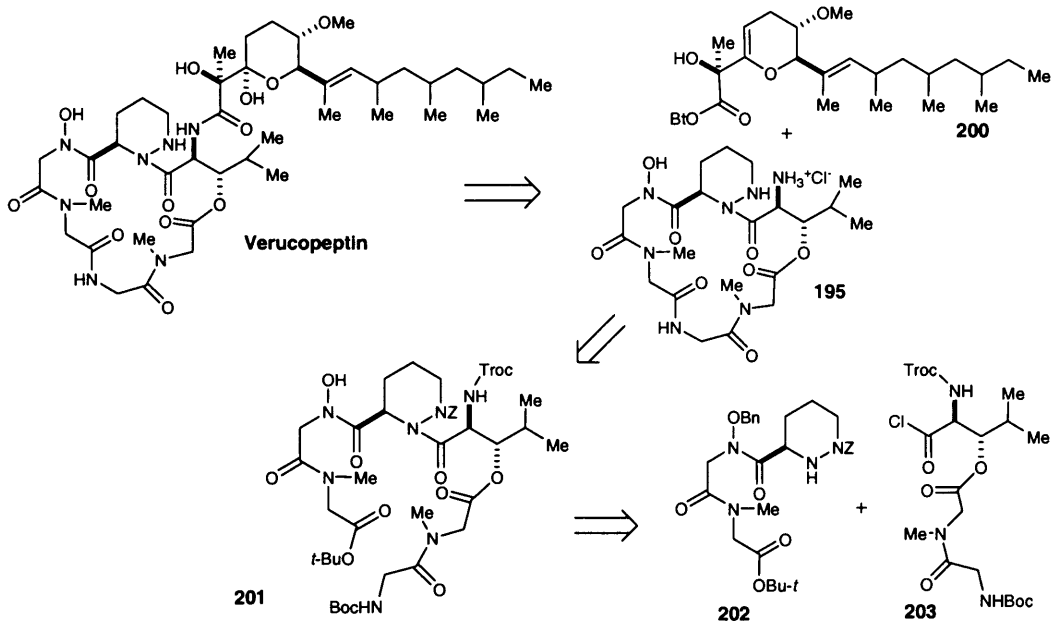
Scheme 46 The A83586C and GE3 acyl side-chains

At the time of writing, I have completed the synthesis of all four cyclodepsipeptide rings shown in Scheme 44, and isolated them as their hydrochloride salts. Moreover the synthesis of three of these has been achieved on gram-scale. We have also successfully completed the synthesis of two natural product analogues using the known A83586C benzotriazole activated ester **47**, and have subsequently submitted them for biological testing. Described in this chapter are all the synthetic routes towards these target molecules and our attempts at synthesising the GE3 acyl side-chain.

4.1 Synthesis of the Verucopeptin Cyclodepsipeptide Ring

The absolute stereochemistry of verucopeptin is still undetermined and as a consequence there are eight diastereomeric possibilities for the cyclodepsipeptide sector. The fact that all

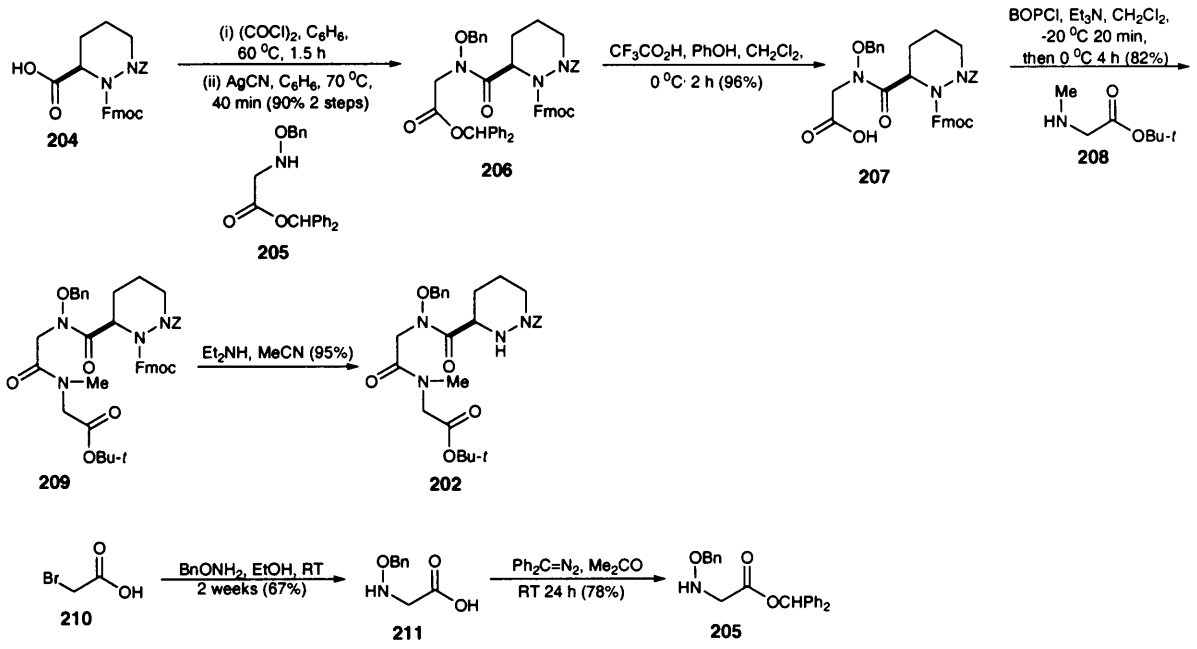
previously identified members of this group possess a (3*R*)-piperazic acid unit connected to a (2*S*, 3*S*)-3-hydroxyleucine residue, strongly suggested that this stereochemical arrangement was also present in verucopeptin. With this in mind our attention centered on the asymmetric total synthesis of diastereoisomer **195**. Our initial plan was to attempt the [3+3]-fragment condensation of protected tripeptides **202** and **203** (Scheme 47) and macrolactamise between the terminal sarcosine and glycine residues to close the 19-membered macrolactone ring. This position was chosen for macrolactamisation as it united the most nucleophilic and the least sterically encumbered residues. Global deprotection would then provide our desired cyclodepsipeptide **195**, ready for chemoselective coupling to a range of activated esters or acid chlorides. Use of pyrans of general structure **200** could potentially allow the total synthesis of verucopeptin itself.



Scheme 47 Retrosynthetic analysis of verucopeptin

The starting point for the synthesis of the orthogonally protected tripeptide **202** (Scheme 48) was known (3*S*)-*N*(1)-*Z*-*N*(2)-Fmoc-piperazic acid **204**. [91] Acid **204** was transformed into dipeptide **206** by first converting it to the acid chloride and coupling it with *N*-benzyloxyglycine diphenylmethyl ester **205** under silver cyanide assisted amidation conditions. The dipeptide **206**

was isolated as a white foam in excellent yield (90%, 2 steps). The diphenylmethyl ester protecting group[107] of **206** was then cleaved with TFA in CH_2Cl_2 at 0^0C in the presence of 1.5 equivalents of PhOH. The latter functioned as a scavenger of the diphenylmethyl cation by-product, allowing acid **207** to be obtained cleanly.

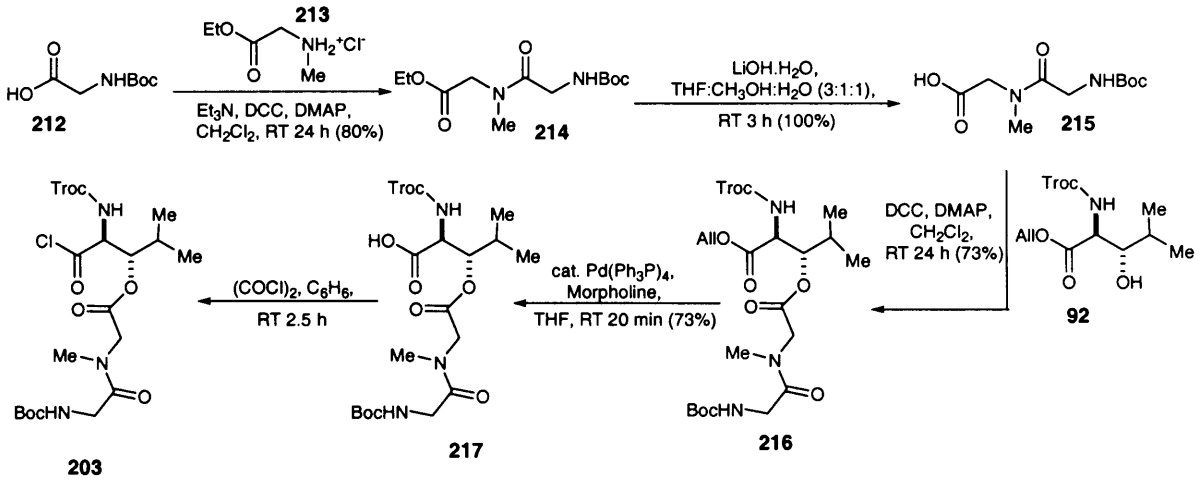


Scheme 48 Synthesis of tripeptide **202**

N-Benzyloxyglycine **205** was synthesised according to the method prescribed by Kolasa and Chimiak,[108] which involves liberating *O*-benzyl-hydroxylamine from its hydrochloride salt using one equivalent of triethylamine, and then stirring an excess of the free amine with bromoacetic acid **210** in the presence of ethanol for 2 weeks at room temperature. This affords **211** as a pure white solid in 67% yield for the 2 steps. This was converted to diphenylmethyl ester **205** by stirring with 1.3 equivalents of diphenyldiazomethane in acetone at room temperature for 24 h. Despite the reaction mixture containing a number of decomposition products of the diphenyldiazomethane, the *N*-benzyloxyglycine diphenylmethyl ester **205** could be purified successfully *via* SiO_2 flash chromatography and was obtained as a colourless oil.

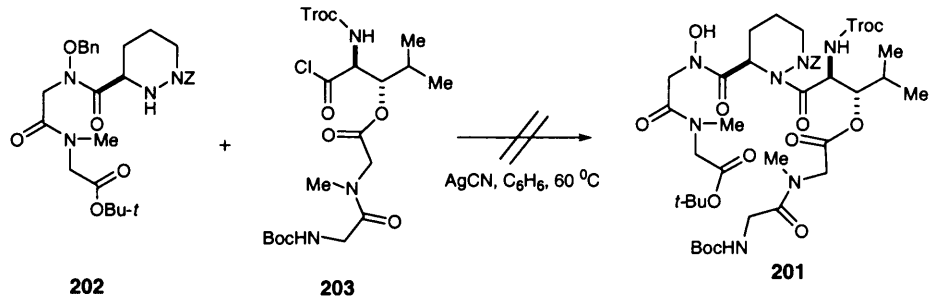
The final coupling in this sequence was between acid **207** and the commercially available *tert*-butyl ester of sarcosine **208**. It was achieved using bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl)[109] as the peptide coupling reagent and triethylamine as base in CH_2Cl_2 at 0 ^{0}C, and gave the tripeptide **209** as a white foam in 82% yield. The requisite tripeptide **202** was obtained after removal of the base labile Fmoc group from **209** with a 1:1 mixture of diethylamine and acetonitrile. The 500 MHz ^{1}H NMR and 125 MHz ^{13}C NMR spectra of the purified tripeptide **202** were difficult to assign due to extensive broadening of the signals which was the result of restricted rotation about the various amide bonds. Nevertheless, the FAB HRMS (High Resolution Mass Spectrum) corroborated the identity of these peptides, it showing an $(M+Na)^+$ peak at *m/e* 577.2660 which compared favourably with the calculated theoretical value of 577.2638 (Calcd. for: $C_{29}H_{38}O_7N_4Na$ $(M+Na)^+$).

The second fragment required for the implementation of our proposed [3+3]-fragment condensation reaction was created according to Scheme 49. Dipeptide **215**[110, 111] was synthesised in two steps by coupling commercially available Boc-glycine **212** with the hydrochloride salt of sarcosine ethyl ester **213** under standard peptide coupling conditions of DCC, catalytic DMAP, and one equivalent of triethylamine to liberate the ethyl ester of sarcosine from its hydrochloride salt *in situ*. The resulting dipeptide **214** was purified by SiO_2 flash chromatography and the ethyl ester hydrolysed using lithium hydroxide monohydrate to give dipeptide **215**. The latter was coupled to the known *N*-Troc-(2*S*, 3*S*)-3-hydroxyleucine allyl ester **92**[94] using DCC and DMAP to form depsipeptide **216** as a viscous oil in 73% yield. The final steps of this sequence were *O*-deallylation[112] of **216** with catalytic $(Ph_3P)_4Pd$ and morpholine in THF (73% yield), and conversion of acid **217** to the acid chloride **203** using excess oxalyl chloride. The acid chloride **203** was never stored but used immediately for the next reaction.



Scheme 49 Synthesis of ester **203**

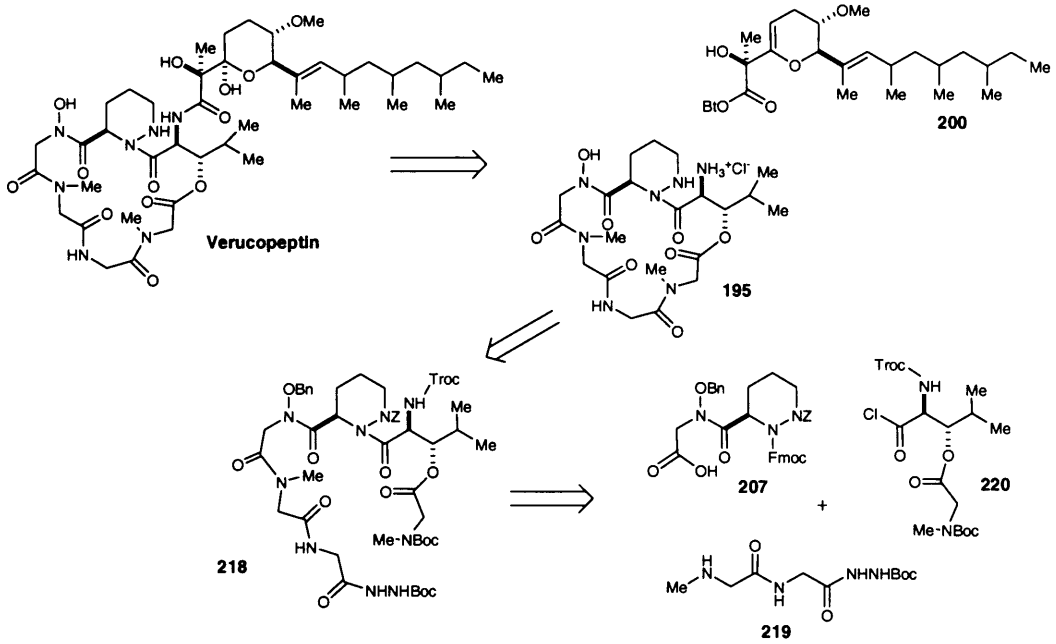
Having completed the synthesis of the two fragments **202** and **203**, we were now in a position to attempt the key [3+3] coupling reaction (Scheme 50). Both components were now heated in the presence of silver cyanide and benzene at 70 ^{0}C for 5 min. Unfortunately after a number of attempts and alterations to the reaction conditions, no reaction appeared to be taking place. The underlying reason for this is unclear. However, we were attempting to synthesise a very elaborate and possibly structurally unstable acid chloride **203**, which could be degrading before a reaction with tripeptide **202** could take place.



Scheme 50 Attempted [3+3]-fragment condensation

Given the poor outcome in the aforementioned [3+3]–fragment condensation we next elected to investigate a [2+2+2]–fragment condensation strategy similar to that used in the A83586C venture. We were attracted to such an approach for building **218**, as [3+3] approaches

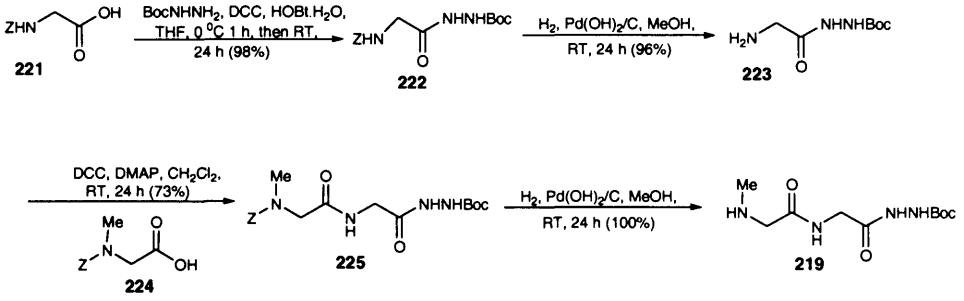
had also proven ineffective in the A83586C venture, whereas the [2+2+2] approach had proven successful. Our proposed [2+2+2]-fragment condensation strategy is depicted in Scheme 51. It was envisioned that cyclodepsipeptide **195** could be accessed from hexadepsipeptide **218** following acid deprotection of the Boc groups and macrolactamisation between the terminal sarcosine and glycine residues. Fragments **207**, **219** and **220** were those required to conduct the [2+2+2]-fragment condensation and provide hexadepsipeptide **218**.



Scheme 51 Revised verucopeptin retrosynthetic strategy

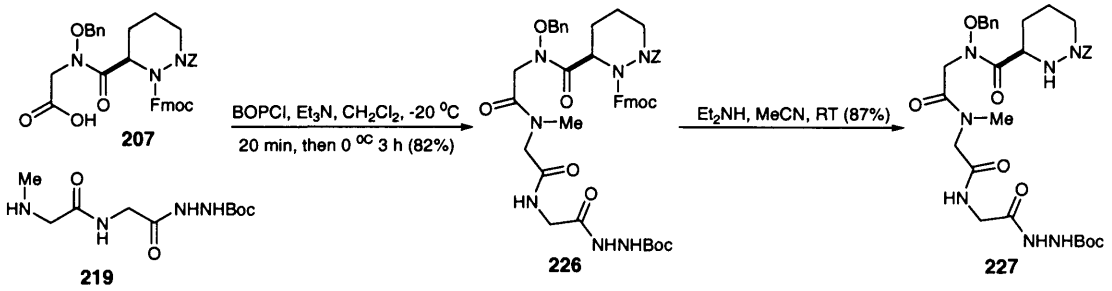
Dipeptide **219** was formed by a 4 step sequence (Scheme 52) that commenced with the protection of commercially available *N*-carboxybenzyl-glycine **221** with *t*-butyl carbazate. Hydrogenolytic removal of the *N*-(Z) group from **222** with Pearlman's catalyst[113] (20% palladium hydroxide on carbon), yielded the *t*-butyl carbazate of glycine **223**, which coupled under standard DCC/DMAP conditions with *N*-(Z)-sarcosine **224** to produce dipeptide **225** in 73% yield after purification by SiO_2 flash chromatography. The required dipeptide **219** was obtained by hydrogenolytically cleaving the *Z* group from **225**, again, utilizing Pearlman's catalyst. Dipeptide **219** was not purified at this stage. Filtration of the reaction mixture through Celite, to remove the palladium residues and concentration *in vacuo* of the filtrate afforded **219** in essentially pure form.

The *t*-butyl carbazate protecting group was selected in preference to a standard ester group to circumvent the problem of forming a diketopiperazine during the final hydrogenolytic removal of the Z group from **225**.



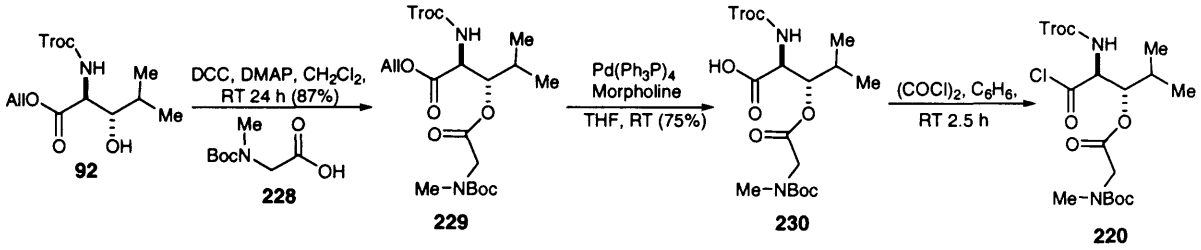
Scheme 52 Synthesis of dipeptide **219**

The coupling of **207** and **219** proceeded efficiently when conducted with BOP-Cl and Et_3N at low temperature in CH_2Cl_2. The tetrapeptide **226** was isolated in 82% yield (Scheme 53). Initial attempts at this particular coupling reaction were unsuccessful. The eventual source of the problem was tracked down to the quality of the BOP-Cl reagent used. In this regard, it was always important to use a fresh batch of BOP-Cl to achieve clean reactions in high yields. Fmoc cleavage from **226** using a 1:1 mixture of diethylamine and acetonitrile then provided **227**. Despite the high polarity of this tetrapeptide we were able to purify it by SiO_2 flash chromatography using 1:1 hexanes/EtOAc as eluant to remove the Fmoc fragments and then 10% MeOH/EtOAc to elute the product.



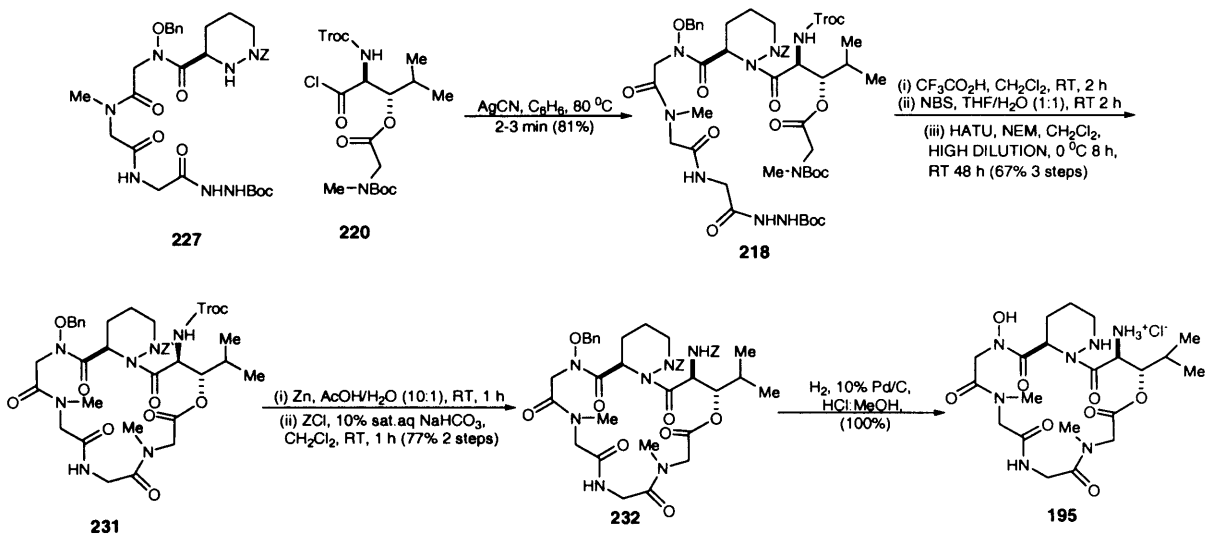
Scheme 53 Synthesis of tetrapeptide **227**

The last fragment to be assembled was acid chloride **220** (Scheme 54). This was prepared via a DCC/DMAP mediated esterification between *N*-Boc sarcosine **228** and **92**. The ensuing depsipeptide **229** was then subjected to an *O*-deallylation reaction with cat. $Pd(PPh_3)_4$ and morpholine as the allyl cation scavenger. The acid **230** was then converted to the acid chloride **220** by treatment with excess oxalyl chloride in benzene.



Scheme 54 Synthesis of acid chloride **220**

With the tetrapeptide **227** and acid chloride **220** fragments assembled, we were now ready to attempt the key [4+2] coupling reaction (Scheme 55). Fragment condensation was achieved by heating acid chloride **220** with tetrapeptide **227** in C_6H_6 at 80 ^{0}C in the presence of silver cyanide for 2-3 min. The faster moving hexadepsipeptide **218** was isolated in 81% yield for the two steps. Prolonging the reaction beyond this time frame led to a serious diminution in the yield of hexadepsipeptide **218** product, due to *in situ* degradation of the product. Presumably, this decomposition was the result of the Troc group reacting with the Ag^+ that was present in the reaction mixture.



Scheme 55 Synthesis of the verucopeptin cyclodepsipeptide salt **195**

With a synthesis of the linear hexadepsipeptide **218** now accomplished we proceeded towards the all important macrolactamization step and eventual completion of our asymmetric total synthesis of the verucopeptin cyclodepsipeptide core **195**. Initially we attempted an *N*-(Troc) to *N*-(Z) group interconversion as we had in the synthesis of A83586C. Hence **218** was subjected to Zn dust in 10:1 $AcOH/H_2O$ and the reaction proceeded cleanly by TLC. The crude amine was then dissolved in CH_2Cl_2 and at 0 °C 10% $NaHCO_3$ and Z-Cl were added simultaneously. Unfortunately at this stage the TLC analysis showed multiple spots. We believed that under the basic conditions an *O*- to *N*-acyl migration might be taking place. In light of this we felt it best to keep with the *N*-Troc protecting group and cleave it at a later stage in the synthesis.

Therefore the Boc groups were detached from the two terminal residues of the linear hexadepsipeptide **218** by treatment with excess trifluoroacetic acid. Notwithstanding the terminal sarcosine amine, the glycinyl acid hydrazide group could be chemoselectively oxidized with NBS in 1:1 THF/H_2O to give the acid. Presumably, the NBS converts the acyl hydrazine unit into a highly reactive acyl diazene, which is instantly intercepted by the excess of water that is present to form the acid. Macrolactamisation was performed with 10 equivalents of HATU (*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*-tetramethyluronium hexafluorophosphate) and 13.5 equivalents of NEM (*N*-

ethylmorpholine) in CH_2Cl_2 under conditions of very high dilution (0.0004 M) to give cyclodepsipeptide **231**. In the macrolactamization a solution of the hexadepsipeptide and NEM in dry CH_2Cl_2 were added dropwise to an ice-cold suspension of HATU in dry CH_2Cl_2. After the addition, the reaction was allowed to warm to RT and left to stir for 48 h. Concentration of the reaction mixture *in vacuo*, acid/base workup, and purification by SiO_2 flash chromatography afforded the protected cyclodepsipeptide **231** in an overall excellent yield of 67% for the three steps. The product stained golden brown in anisaldehyde/H_2SO_4 stain on TLC.

High dilution conditions were found to be essential for clean cyclizations as attempts to run the reaction at higher concentrations (0.05 M, 0.01M, 0.001 M), always gave two spots by TLC, a baseline spot and a slightly higher running spot (CH_2Cl_2/MeOH 40:1). Isolation of the baseline spot and ^1H NMR analysis gave a spectrum that were similar to the protected cyclodepsipeptide **231** but the peaks were very much broadened. This was shown to be the dimerization product.

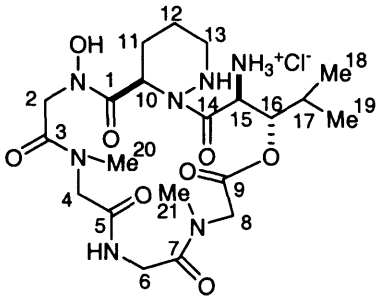
At this stage we were ready to investigate the *N*-Troc to *N*-Z group interconversion on **231**. The *N*-Troc group of the hydroxyleucine moiety was cleaved with Zn dust in aqueous acetic acid. To facilitate the final purification of **195** and help remove all the Zn salts, we temporarily capped the crude product amine with a *Z*-group using benzyl chloroformate and 10% aqueous $NaHCO_3$. This two step procedure worked well and very cleanly to give cyclodepsipeptide **232** as a white solid in an overall 77% yield. Hydrogenolytic cleavage of all the benzyl protecting groups from cyclodepsipeptide **232** was best achieved using 10% wet palladium on carbon in the presence of one equivalent of HCl in MeOH. The mildly acidic conditions of this step were designed to protonate the hydroxyleucine amine as it was liberated and prohibit *O*- to *N*- acyl migration in the final product. This side-reaction is known to be problematic in such systems during prolonged hydrogenations. The verucopeptin cyclodepsipeptide salt **195** was isolated as a white solid in quantitative yield.[114]

When we analysed the 500 MHz ^{1}H NMR spectrum of the crude salt in CD_3OD we observed a fairly clean spectrum that corresponded essentially to a single diastereoisomer. It appears that the CD_3OD locks the cyclodepsipeptide into a single conformation. When the same sample was run in D_2O, its ^{1}H NMR was much more complex and showed the presence of several interconverting forms. Consequently, we recommend analysis of **195** in CD_3OD when judging product purity.

The structure of **195** was further confirmed by its FAB HRMS which contained an $(M+H)^+$ peak at *m/e* 514.2600 (Calcd. for: $C_{21}H_{36}O_8N_7$ $(M+H)^+$: 514.2625). The IR spectrum (KBr) showed a strong and broad absorption at 1656 cm^{-1} verifying the peptide linkages, and there was an absorption at 1746 cm^{-1} indicative of a cyclodepsipeptide ester linkage. Additional evidence to support the assigned structure was provided by the room temperature 125 MHz ^{13}C NMR spectra of **195** in MeOD, which clearly showed that it was a single compound and existed as one confomer. Comparisons with the spectra obtained for verucopeptin[7, 8] and 2D NMR methods allowed us to fully assign both the ^{1}H NMR and ^{13}C NMR of **195** (Table 1).

Some of the main signals in the 500 MHz ^{1}H NMR spectrum of **195** were: the two Me groups which appeared as two doublets at δ 1.16 ppm (J = 6.7 Hz) and δ 0.91 ppm (J = 6.7 Hz); and the two *N*-Me groups which appeared as two singlets at δ 3.15 ppm and δ 2.87 ppm. The four methylene groups of the cyclodepsipeptide ring (Positions 2, 4, 6 and 8 in Table 1) each resonated as a pair of doublets. Despite not being able to assign the exact position of each CH_2 group within the ring we were able, via 2D NMR methods and comparison of J coupling values, to match the protons attached to the same carbon.

The 125 MHz ^{13}C NMR spectrum of **195** contains 21 carbon signals including six amide/ester carbonyl carbons (δ ppm 173.7, 172.4, 172.0, 169.9, 169.2, 169.1). The ^{13}C DEPT analysis gave 7 methylenes, 6 quaternary carbons and 8 methine and methyl carbons.

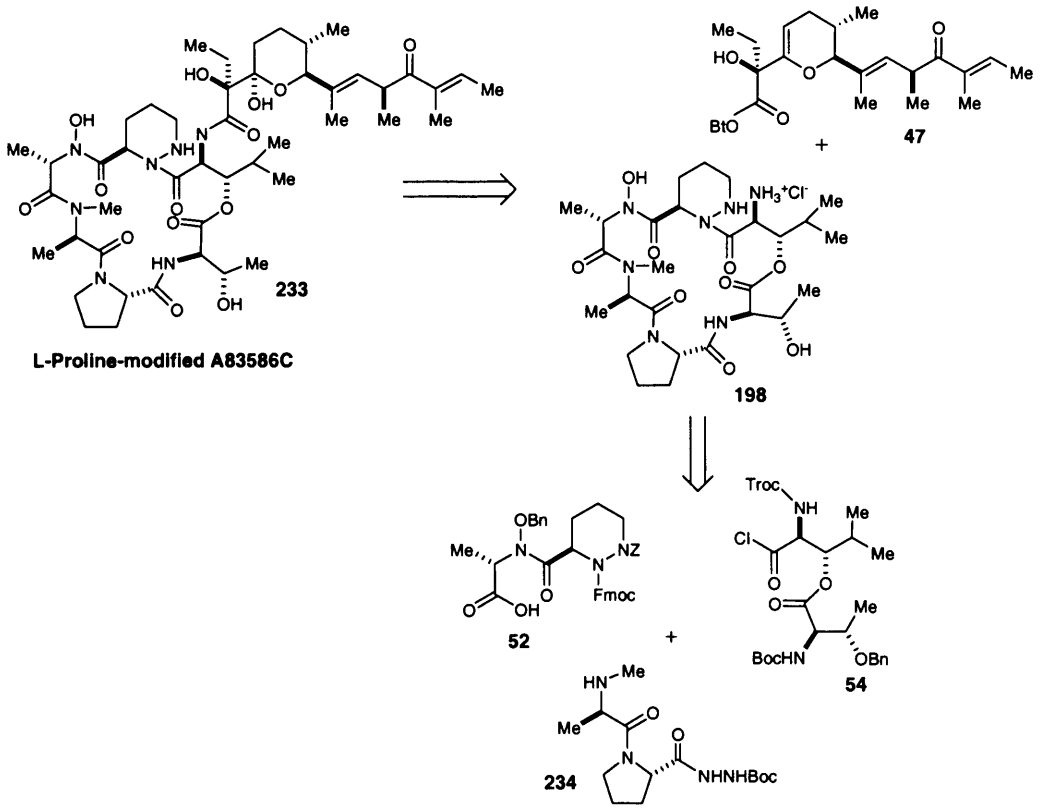


195

Position(s)	125MHZ ^{13}C NMR δ (ppm)	500MHz ^{1}H NMR δ (ppm)
1, 3, 5, 7, 9, 14	173.7, 172.4, 172.0, 169.9, 169.2, 169.1	
2, 4, 6, 8	52.3, 42.7, 52.7, 53.4	5.38 (d, 15.3 Hz, 1H) 3.81 (d, 15.3 Hz, 1H), 5.01 (d, 17.7 Hz, 1H) 3.75 (d, 17.7 Hz, 1H) 4.45 (d, 17.4 Hz, 1H) 3.91 (d, 17.4 Hz, 1H) 4.0 (d, 17.0 Hz, 1H) 3.87 (d, 17.0 Hz, 1H)
10	51.1	5.13 (br d, 5.8 Hz, 1H)
11	24.2	2.18 (br d, 13.7 Hz, 1H), 1.98 (m, 1H)
12	21.8	1.7-1.45 (m, 2H)
13	47.5	3.10 (m, 1H) 2.73 (dt, 12.6 Hz, 3.3 Hz, 1H)
15	50.5	5.27 (br s, 1H)
16	78.4	5.08 (br d, 9.9 Hz, 1H)
17	30.3	1.84 (m, 1H)
18,19	19.2, 19.3	1.16 (d, 6.7 Hz, 3H) 0.91 (d, 6.7 Hz, 3H)
20,21	37.3, 34.9	3.15 (s, 3H) 2.87 (s, 3H)

Table 1 125 MHz ^{13}C and 500 MHz ^{1}H NMR data for **195** in CD_3OD at 298K

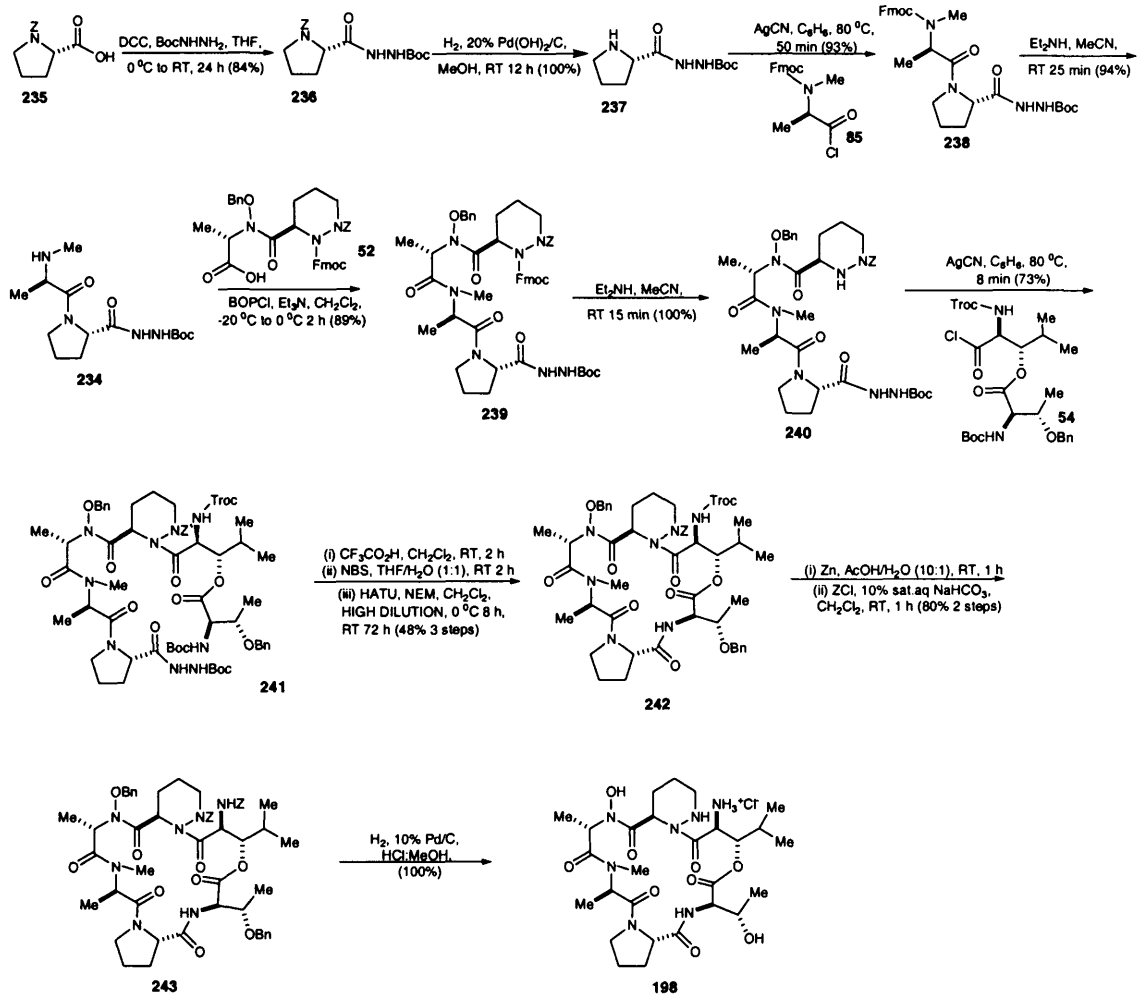
4.2 Synthesis of an L-Proline Modified Mimetic of A83586C



Scheme 56 Retrosynthetic analysis of the L-Pro modified mimetic of A83586C **233**

Our retrosynthetic analysis of **233** is illustrated in Scheme 56 and was founded upon the chemoselective union of activated ester **47** with the cyclodepsipeptide hydrochloride salt **198**. Again the macrolactam **198** would potentially be accessed via our previously successful [2+2+2]-fragment condensation strategy, with the key ring closure being performed between the terminal L-proline and D-threonine residues. This site was selected for ring closure for a number of reasons. The *N*-2 position of piperazic acids is electronically deactivated and sterically shielded when *N*-1 is carbamoylated. The *N*-Me amino residues are also sterically shielded by the Me group and hence not as active as a primary amino group for a macrolactamisation. The hydroxamic acid amine unit is electronically deactivated and hence this is not a good strategic position for ring closure. Finally the presence of the L-proline was predicted to have a positive effect on the macrolactamisation since it would constrain the linear hexadepsipeptide in a position that would bring the acid moiety of

the L-proline into close proximity with the D-threonine amino residue. Fragments **52** and **54** were known from the total asymmetric synthesis of A83586C and so all that was required to implement the [2+2+2]-strategy was dipeptide **234**.



Scheme 57 Synthesis of the L-Pro modified mimetic of A83586C cyclodepsipeptide salt **198**

The starting point for the synthesis of **198** was commercially available *N*-(Z)-L-proline **235** (Scheme 57) which was amidated with *t*-butyl carbazate and DCC to afford carbazate **236** in 84% yield. The benzyloxycarbonyl group of **236** was removed from the product by hydrogenation in methanol with a 20% $Pd(OH)_2$ catalyst to afford the L-proline carbazate **237** in 84% yield for the two steps. Compound **237** was coupled to the known Fmoc-*N*-methyl-D-alanyl-chloride[117] **85** in C_6H_6 at 80°C using silver cyanide as a promoter; this was a very clean reaction giving the faster moving

dipeptide **238** in 93% yield. Fmoc cleavage with Et_2NH in acetonitrile resulted in the amine **234**, which was purified and coupled with known dipeptide **52** utilizing BOP-Cl as an activating agent in the presence of Et_3N at low temperature.

The coupling protocol that was used successfully in the synthesis of the A83586C cyclodepsipeptide proved to be equally successful in this case. To a mixture of one equivalent of each of the dipeptides **52** and **234** in dry CH_2Cl_2 at –20 ^{0}C was added 1.2 equivalents of a fresh batch of BOP-Cl and 2 equivalents of dry Et_3N. The mixture was left to stir at 0 ^{0}C for 1 h taking care not to allow the temperature to rise above 5 ^{0}C; at temperatures above 5 ^{0}C, Et_3N mediated Fmoc cleavage occurs. After acid/base workup and SiO_2 flash chromatography tetrapeptide **239** was obtained in 89% yield as a white foam. Cleavage of the Fmoc grouping from tetrapeptide **239** afforded **240** ready for a [4+2] silver cyanide facilitated condensation with known depsipeptide **54**.

For the [4+2] condensation a solution of 1 equivalent of tetrapeptide **240** in dry C_6H_6 was added at room temperature to 1 equivalent of the freshly prepared acid chloride **54**. To this reaction mixture was added 1.5 equivalents of AgCN, and the reaction was heated at 80 ^{0}C for 8 min with the exclusion of light. It is presumed that the Ag^+ ion coordinates with the Cl of acid chloride **54** enhancing its electrophilic character, at which point the CN^- ion conducts a displacement to form the acyl cyanide that is then attacked nucleophilically by amine **240** to form the amide bond. The reaction mixture was cooled and filtered through Celite to remove the AgCl salt, and the filtrate concentrated *in vacuo* to give the crude product which despite being clean was purified by SiO_2 flash chromatography to give hexadepsipeptide **241** in an encouraging 73% yield.

Conversion of **241** to the macrolactam **242** was very effectively conducted using the three-step protocol developed during our verucopeptin cyclodepsipetide synthesis. Thus, TFA induced Boc-group cleavage from **241**, NBS facilitated oxidation of the acyl hydrazine and high dilution macrocyclisation with HATU/NEM produced the macrolactam **242** in 48% overall yield for the three steps. Acquisition of the target cyclodepsipeptide **198**[118] was accomplished after Troc group

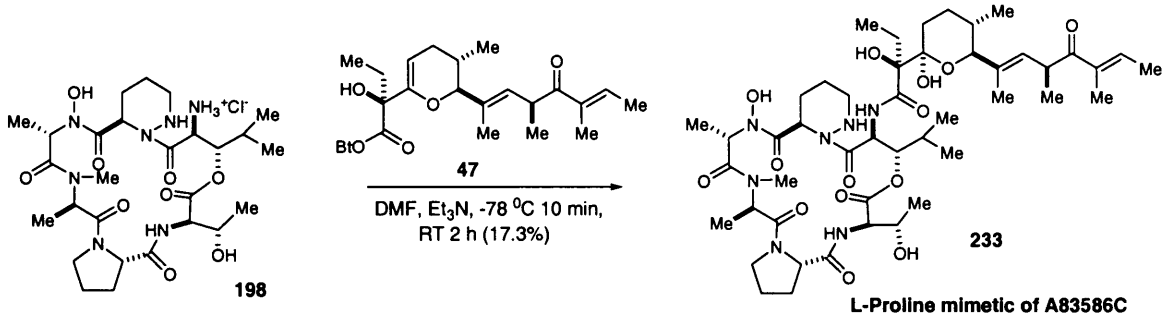
removal with Zn/AcOH, *N*-acylation with benzyl chloroformate and aq. $NaHCO_3$, and finally hydrogenation over Pd/C in the presence of 1 equivalent of HCl in MeOH.

The *N*-Troc for *N*-Z group interconversion proceeded in an excellent overall yield of 80%. Cyclodepsipeptide **242** was subjected to a large excess of Zn dust in 10:1 $AcOH/H_2O$ for 25 min at RT. Filtration through Celite to remove the Zn and concentrated *in vacuo* gave the crude amine, which was immediately capped with a *Z* group by dissolving in CH_2Cl_2 and simultaneously adding 3 equivalents of Z-Cl and 10% aq. $NaHCO_3$. Immediate protection of the hydroxyleucine NH_2 group was important to aid product isolation and also prevent the potential *O*- to *N*-acyl migration. Purification with SiO_2 flash chromatography afforded pure cyclodepsipeptide **243**. This purification was extremely important because we would not be able to purify the final cyclodepsipeptide salt by chromatographic methods later on. Provided precursor **243** was pure, simple filtration of the hydrogenation mixture and concentration of the filtrate would afford virtually clean cyclodepsipeptide salt **198**.

The structure of **198** was confirmed by its FAB HRMS which contained an $(M+H)^+$ peak at *m/e* 612.3338 (Calcd for $C_{27}H_{46}N_7O_9$ $[M+H]^+$, 612.3357), and by its IR spectrum which showed an intense C=O stretching absorption at 1746 cm^{-1} indicative of a cyclodepsipeptide ester linkage. The room temperature 500 MHz ^1H NMR and 125 MHz ^{13}C NMR spectra of **198** in CD_3OD were not as well resolved as the verucopeptin spectra, but nevertheless showed a single compound.

The 500 MHz ^1H NMR of **198** contained five doublets corresponding to the five Me groups which resonated at δ (ppm) 0.90, 1.05, 1.20, 1.30 and 1.35. The *N*-Me group appeared as a singlet at δ 3.0 ppm. The 125 MHz ^{13}C NMR contained 27 carbon signals including six amide/ester carbonyl carbons (δ (ppm) 174.9, 173.1, 172.0, 171.7, 170.8, 168.7), and ^{13}C DEPT analysis confirmed the presence of 6 methylene groups.

With **198** in hand we proceeded to couple this cyclodepsipeptide salt to the A83586C activated ester **47** (Scheme 58). For this final coupling we used the protocol used effectively in the A83586C synthesis. Hence to a solution of 1 equivalent of cyclodepsipeptide salt **198** in dry CH_2Cl_2 under an atmosphere of N_2 was added 1.2 equivalents of activated ester **47**. The reaction mixture was cooled to –78 ^{0}C and a large excess of dry Et_3N added dropwise. The reaction was stirred at –78 ^{0}C for 10 min and then warmed to RT and stirred for about 1.5 h. The reaction was analysed by TLC which seemed messy but following acid/base workup of the reaction mixture the TLC cleaned up enormously to give a golden brown spot when stained with anisaldehyde/H_2SO_4. This was purified by preparative TLC using 30:1 CH_2Cl_2:MeOH as eluant. This afforded the glycal, which was further purified by SiO_2 flash chromatography (hexanes:EtOAc 2:1 then 0:1). The glycal hydrated in $CDCl_3$ to give the final L-proline analogue of A83586C **233** as a white solid in 17.3% yield.



Scheme 58 Final coupling between **198** and **47** to yield target molecule **233**

The structure of **233** was confirmed by its FAB HRMS which contained an $(M+Na)^+$ peak at *m/e* 985.5378 (Calcd for $C_{47}H_{75}N_7O_{14}Na$ $[M+Na]^+$, 985.5348), and by its IR spectrum which showed an intense C=O stretching absorption at 1729 cm^{-1} indicative of a cyclodepsipeptide ester linkage. The 500 MHz ^1H NMR and 125 MHz ^{13}CNMR in $CDCl_3$ at 298K, which showed a single diastereoisomer, were assigned by direct comparisons with the NMR spectra of the A83586C natural product.[2]

The 125 MHz ^{13}C NMR spectrum of **233** contained 47 carbon signals including one ketone carbon (δ 202.9 ppm) and seven amide/ester carbonyl carbons (δ (ppm) 175.3, 175.0, 174.4, 171.2, 170.5, 170.0, 168.8). The ^{13}C DEPT analysis gave 9 methylenes, 12 (4+8carbonyls) quaternary carbons and 26 methine and methyl carbons. The quaternary carbon peak at δ 99.6 ppm was indicative of hydration of the glycal. The four carbon atoms of the two olefinic bonds of the acyl side chain were also apparent with the two quaternary sp^2 carbons resonating at δ 137.5 ppm and 132.8 ppm, and the other two sp^2 carbons resonating at δ 136.7 ppm and 129.4 ppm.

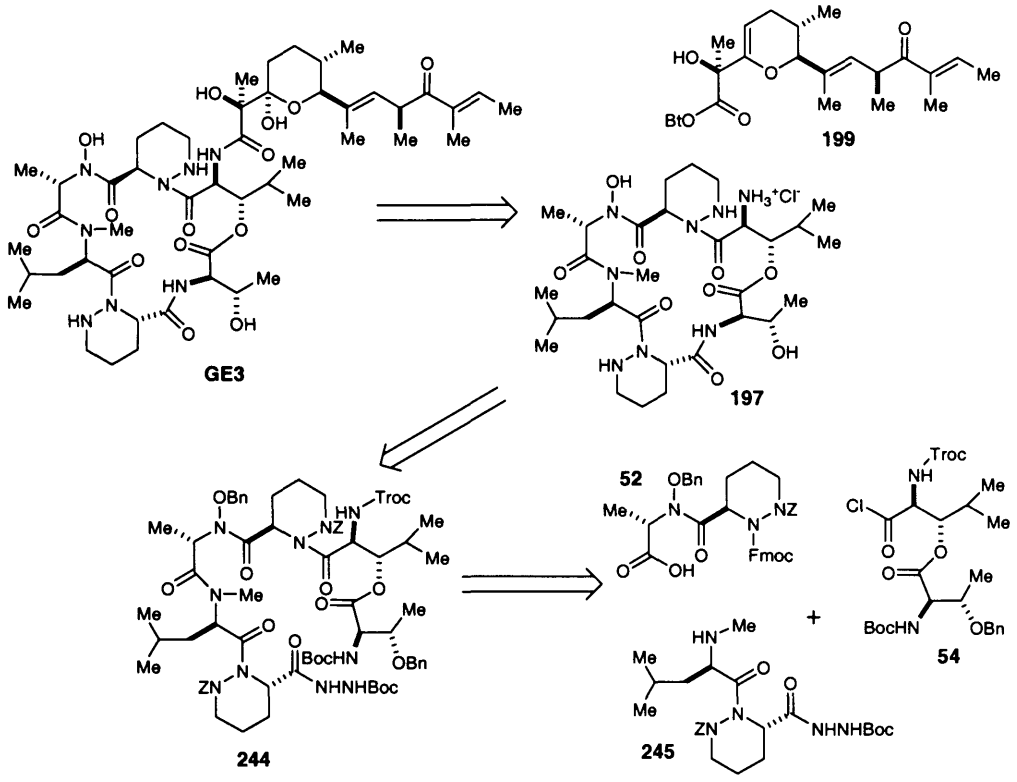
The ^1H NMR spectrum of **233** contained a low field shifted signal (δ 9.25 ppm, br s) assignable to the *N*-hydroxy proton. Also apparent were 11 methyl signals which resonated at δ 1.83 ppm (dd, J = 7.1 Hz, 1.0 Hz), 1.74 ppm (br s), 1.55 ppm (d, J = 1.2 Hz), 1.47 ppm (d, J = 7.1 Hz), 1.32 ppm (d, J = 6.9 Hz), 1.09 ppm (d, J = 7.0 Hz), 1.04 ppm (d, J = 6.5 Hz), 0.82 ppm (t, J = 7.2 Hz), 0.70 ppm (d, J = 6.8 Hz), 0.68 ppm (d, J = 6.6 Hz) and 0.63 ppm (d, J = 7.2 Hz). The *N*-Me peak resonated as a singlet at δ 3.11 ppm. The OH of the lactol was present as a broad singlet at δ 6.29 ppm. Some of the other main peaks were: the NH of the hydroxy-leucine residue which appeared as a doublet at δ 8.20 ppm (J = 10.9 Hz); the α proton of the N-OH-alanine residue which resonated as a quartet at δ 5.20 ppm (J = 7.2 Hz); and the two olefinic protons which appeared at δ 6.70 ppm (dq, J = 7.0 Hz, 1.3 Hz) and δ 5.60 ppm (dd, J = 9.1 Hz, 1.3 Hz).

4.3 Synthesis of the GE3 Cyclodepsipeptide Ring

As discussed previously in Chapter 1, GE3 is structurally identical to A83586C except for two positions. Although seemingly minor, these two changes have significant implications for the synthesis of GE3, with modified tactics being needed for its assembly.

Our retrosynthetic plan for the GE3 cyclodepsipeptide (Scheme 59), is based upon the [2+2+2]-fragment condensation method that had proved so effective in the construction of all our previously synthesised cyclodepsipeptide cores. The site chosen for ring closure was that between

the (3*S*)-*N*(1)-*Z*-*N*(2)-piperazic acid and the D-threonine amino residue, for the same reasons explained above.

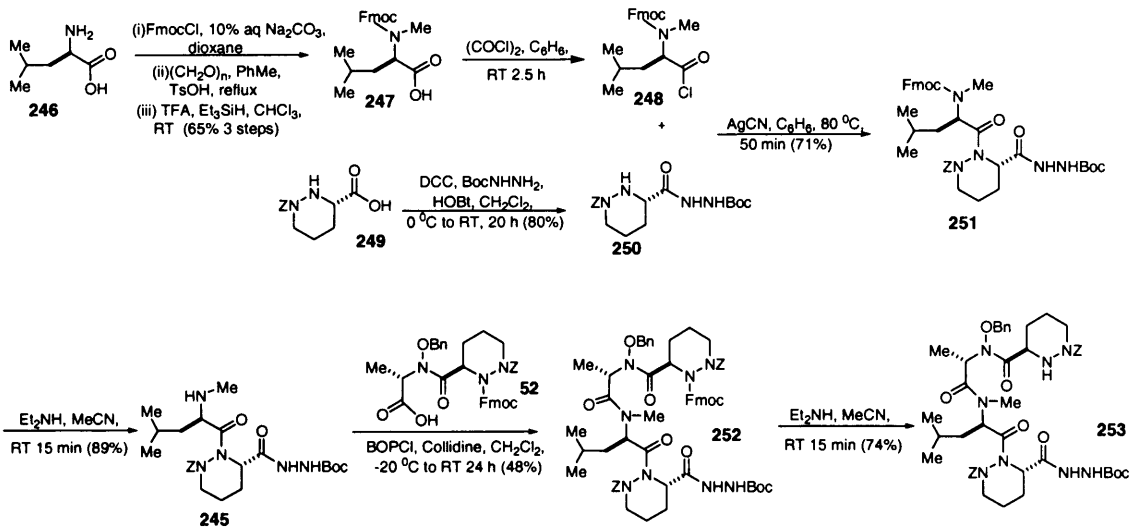


Scheme 59 Retrosynthetic analysis of GE3

Since dipeptide **52** and depsipeptide **54** were known intermediates, our attention focused upon the formation of dipeptide **245**. We started with known (3*S*)-*N*(1)-Z-piperazic acid **249** (Scheme 60) which was converted to the acyl hydrazide **250** by treatment with $BocNHNH_2$, DCC and HOBt in THF. This reaction did not require protection of the *N*-(2) of (3*S*)-*N*(1)-Z-piperazic acid **249**, which is sufficiently electronically deactivated not to interfere with the reaction. The product acyl hydrazide **250** despite being very polar could be purified by SiO_2 flash chromatography using EtOAc as eluant; it being obtained as a white foam in 80% yield. To obtain target dipeptide **251**, acyl hydrazide **250** had to be chemoselectively coupled with Fmoc-*N*-methyl-D-leucinyl chloride **248**.

Fmoc-*N*-methyl-D-leucinoyl chloride **248** was synthesised in four straightforward steps from commercially available D-Leucine **246**. The amine functionality of D-Leucine **246** was protected with an Fmoc group by adding a solution of FmocCl in dioxane at 0 ^{0}C, to a stirred solution of D-leucine **246** and 10% aq. $NaHCO_3$, and then stirring at RT for 2 h. Acidification of the aqueous layer, extraction with EtOAc, and concentration *in vacuo* of the dried organic layers afforded the crude *N*-Fmoc-D-leucine as a white foam. This crude foam was then *N*-methylated in two steps. Initial treatment with paraformaldehyde and catalytic TsOH in refluxing PhMe with azeotropic removal of H_2O, afforded the crude aminoacetal which underwent ionic reduction with TFA/Me_3SiH in high yield; the Fmoc-*N*-methyl-D-leucine **247** was obtained in pure condition, in an overall 65% yield, simply by recrystallisation.

Fmoc-*N*-methyl-D-leucine **247** was converted to the acid chloride **248** by treatment with excess oxalyl chloride in benzene at RT for 2.5 h. After removal of the excess reagent *in vacuo*, the crude acid chloride **248** was formed as a yellow foam and was used immediately for the following coupling step. Hence a solution of 1 equivalent of amine **250** in dry C_6H_6, and 1.5 equivalents of AgCN were added to **248** and the reactants were heated at 80 ^{0}C for 45 min. A faster moving product was formed according to TLC analysis (Hexanes:EtOAc 1:1), that stained golden brown with anisaldehyde/H_2SO_4. Simple filtration of the reaction mixture through Celite, concentration *in vacuo*, and SiO_2 flash chromatography afforded the pure dipeptide **251** as a white foam in 71% yield. Finally the Fmoc group was detached from **251** to give the coupling precursor **245** ready for our trusted BOPCl/Et_3N mediated [2+2]-coupling reaction with dipeptide **52**.

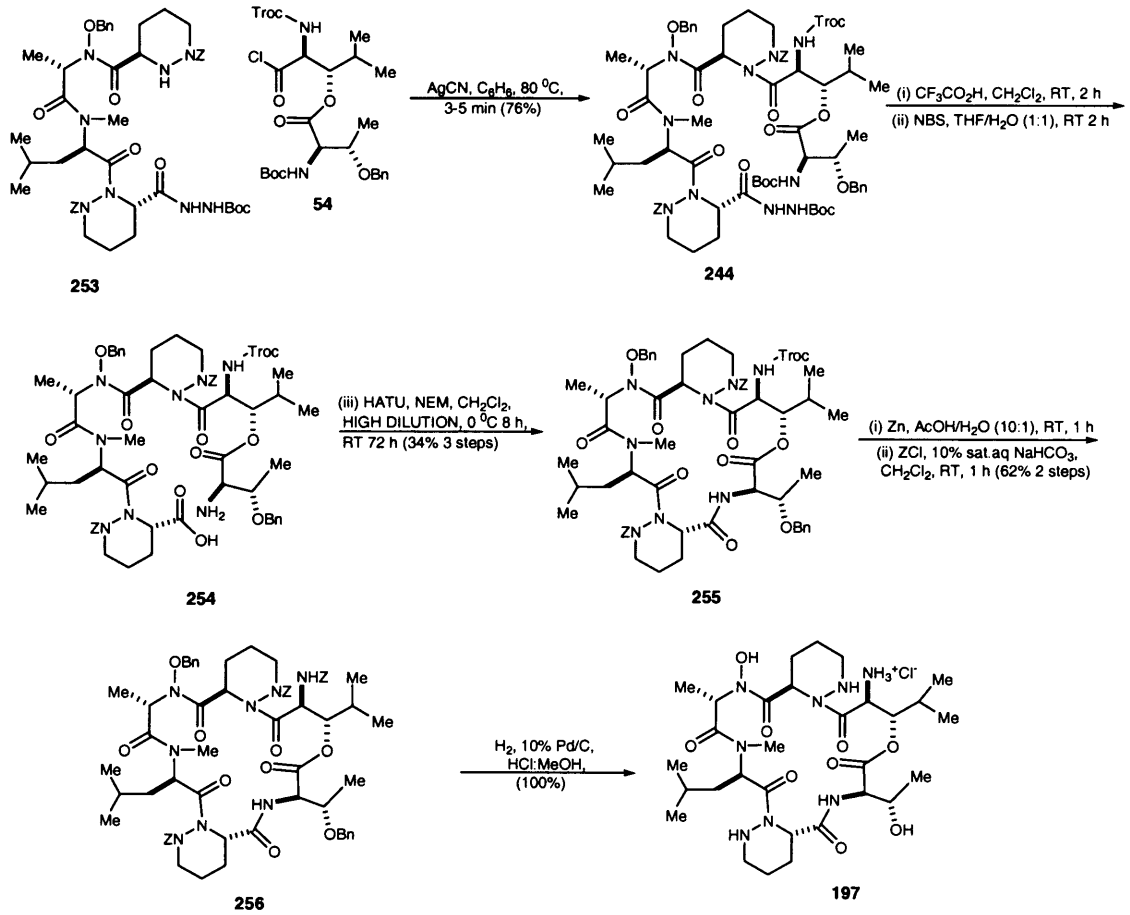


Scheme 60 Synthesis of tetrapeptide **253**

The BOPCl/Et_3N system that had performed well in our previous syntheses failed to deliver the required tetrapeptide **252** in this system. Previously, this reaction had provided clean TLCs with a clear product spot in our syntheses of other tetrapeptides, but gave a very messy TLC appearance in the case of dipeptides **52** and **245**. We attribute this to base-mediated breakdown of the intermediary mixed anhydride brought about by slower coupling to the more hindered **245** fragment. The latter contains a large *sec*-butyl group α to the MeNH. We believed that we required a base that would be sufficiently basic to mediate the acylation but bulky enough to prevent base catalyzed decomposition of the mixed acyl phosphonic acid anhydride and the *N*-Fmoc protecting group. To this end, freshly distilled collidine was screened and found to be beneficial.

Thus to 1 equivalent of acid **52** in dry CH_2Cl_2 at –20 °C and under N_2, was added 1.1 equivalents of distilled collidine and 1.2 equivalents of BOP-Cl and the reaction left to stir at –20 °C for 20 min to allow the formation of the mixed phosphinic anhydride. Then 1 equivalent of amine **245** and another 1.1 equivalents of distilled collidine were added, and the reaction was warmed to RT and stirred for 16 h. These conditions allowed the requisite tetrapeptide **252** to be formed in an

acceptable 48% yield. The beneficial efficacy of collidine upon the course of this coupling was central to completion of the synthesis of the final GE3 cyclodepsipeptide core **197**. Presumably the greater steric bulk of collidine compared with Et_3N helped to prevent it from initiating base-catalyzed decomposition of the intermediary mixed anhydride derived from **52**, and prevent Fmoc group cleavage at temperatures above 0 ^{0}C. The latter was especially problematic when Et_3N was employed as the base, due to the much slower rate of coupling of **245** and **52**. This was as a consequence of enhanced steric hindrance around the *N*-methyl-D-leucine nitrogen in **245**, compared with its A83586C *N*-methyl-D-alanine dipeptide counterpart. The reaction sequence was completed by a standard Fmoc group cleavage with excess Et_2NH in MeCN, to yield tetrapeptide **253** in 74% yield. It is noteworthy that using our above developed synthesis we were able to produce in excess of 20 g of tetrapeptide **253**.



Scheme 61 Synthesis of the GE3 cyclodepsipeptide salt **197**

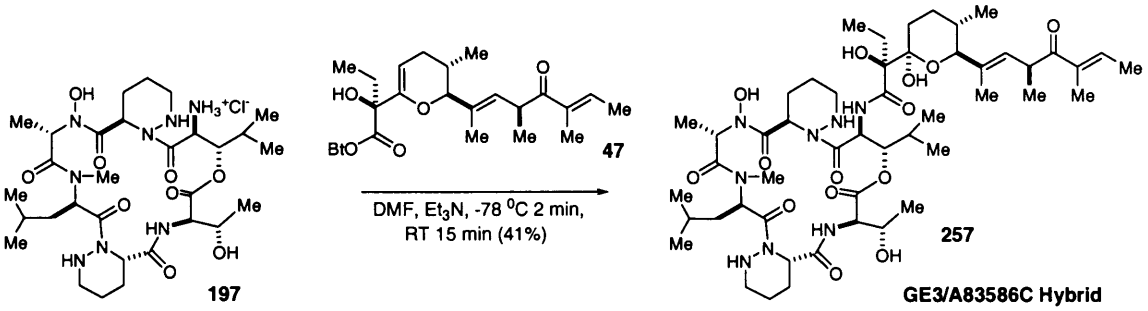
The culmination of this synthetic venture is shown schematically in Scheme 61. Silver cyanide mediated [4+2] coupling of **253** with previously prepared acid chloride **54** led to the linear hexadepsipeptide **244** in a good 76% yield. Conversion of this precursor into **255** was achieved by our standard three-step protocol of TFA induced Boc-group cleavage, NBS facilitated oxidation of the acyl hydrazine, and high dilution macrocyclisation with HATU/NEM. Thus starting with 19 g of linear hexadepsipeptide **244** we isolated 15 g of crude macrolactam precursor **254**. This was subjected in three 5g batches to the above 3 step protocol, the macrolactamisation requiring 8.8 L of dry CH_2Cl_2 on each occasion. After SiO_2 flash chromatography, the 15 g of crude macrolactam precursor **254** afforded a combined total of 5 g of macrolactam **255**. This corresponded a 34% overall yield for the 3 steps which was lower than the 67% yield seen with verucopeptin and 48% for the L-proline analogue cyclodepsipeptide. The GE3 cyclisation yielded two products that were visible by TLC after workup and concentration. This was not the case for the verucopeptin or L-proline analogue ring closures, where only a single product was formed. The higher running compound was the correct product (confirmed by FAB HRMS), whereas we are still not certain what the slower running product is. Nevertheless the two molecules could be separated by SiO_2 flash chromatography.

The final target GE3 cyclodepsipeptide ring[119] **197** was isolated after subjecting the macrolactam **255** to a further three steps. As previously at this stage, the *N*-Troc group was cleaved with an excess of Zn in 10:1 $AcOH/H_2O$ and the isolated crude amine was capped on the hydroxyleucine nitrogen with a *Z* group by reacting it with 3 equivalents of Z-Cl and 10% aq. $NaHCO_3$ in CH_2Cl_2. This afforded cyclodepsipeptide **256** in 62% yield for the two steps. The global deprotection of the 3 g of **256** that had been prepared was achieved by stirring it vigorously with 1 equivalent of HCl in MeOH and 10% wet Pd/C under an atmosphere of H_2 for 24 h. The reaction mixture was then filtered through Celite and concentrated *in vacuo* to afford the GE3 cyclodepsipeptide salt **197** as a white solid in quantitative yield (1.7g).

The structure of **197** was confirmed by its FAB HRMS which contained an $(M+H)^+$ ion at *m/e* 669.3962 (Calcd. for: $C_{30}H_{53}O_9N_8$ $(M+H)^+$: 669.3936). The IR spectrum (KBr) showed a strong broad absorption at 1747 cm^{-1} indicative of a cyclodepsipeptide ester linkage. The room temperature 500 MHz ^1H NMR and 125 MHz ^{13}C NMR spectra of **197** in CD_3OD were not as well resolved as the verucopeptin spectra, presumably due to the presence of rotamers. Nevertheless we proceeded to couple this compound to the A83586C activated ester **47** (Scheme 62)

With a substantial amount of the GE3 cyclodepsipeptide salt **197** and the A83586C activated ester **47** we were in a position to attempt a final coupling reaction to synthesise the A83586C-GE3 hybrid **257**. We first conducted the reaction as for the L-proline analogue of A83586C, by mixing the two components at –78 ^0C with an excess of Et_3N in CH_2Cl_2 and allowing to warm to RT; yields of product were not especially good.

During the final steps of the L-156,602 synthesis, Caldwell and Durette coupled the linear protected hexadepsipeptide **12** to hydroxybenzotriazole ester **1** in DMF, and this step proceeded well in 56% yield (Scheme 10). Thus we decided to attempt our coupling in DMF and to reduce the amount of Et_3N base to 2 equivalents. Because the amount of Et_3N to be used was minimal we made up a stock solution of this in DMF. Accordingly to 1 equivalent of GE3 cyclodepsipeptide salt **197** and 1 equivalent of A83586C activated ester **47** at –78 ^0C was added 2 equivalents of Et_3N in dry DMF. This modification to the reaction conditions greatly enhanced the appearance of the reaction TLC (developed with anisaldehyde), it giving two major products: a golden spot with a slightly higher running purple spot as in a figure of eight (hydrated activated ester **257** and glycal). Preparative TLC using 20:1 CH_2Cl_2/MeOH as eluant and SiO_2 flash chromatography (hexanes:EtOAc 2:1 then 0:1), afforded **257** following hydration in $CDCl_3$. The yield of **257** was 41%, isolated as a white foam. By this procedure 77 mg of **257** was prepared from 130 mg of **197** and 86 mg of **47**.

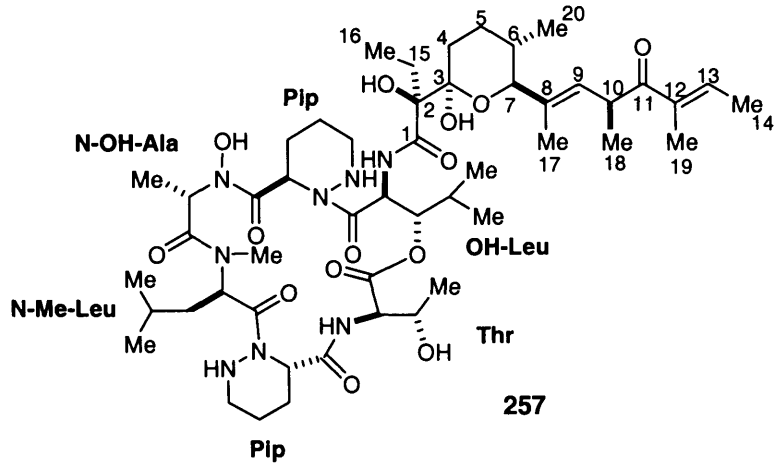


Scheme 62 Final coupling between **197** and **47** to yield target molecule **257**

The structure of **257** was confirmed by its FAB HRMS which contained an $(M+H)^+$ ion at *m/e* 1041.5862 (Calcd. for: $C_{50}H_{82}O_{14}N_8Na$ $(M+Na)^+$: 1041.5848). The IR spectrum (KBr) showed a strong broad absorption at 1729 cm^{-1} indicative of a cyclodepsipeptide ester linkage. The ^1H NMR and ^{13}C NMR spectra of **257** were virtually identical to those of the GE3 natural product[11] and so we were able to fully assign the spectra (Table 2).

The 125 MHz ^{13}C NMR spectrum of **257** contains 50 carbon signals including one ketone carbon (δ 203.0 ppm) and seven amide/ester carbonyl carbons (δ (ppm) 175.3, 174.1, 173.6, 172.2, 170.9, 170.3, 169.5). The ^{13}C DEPT analysis gave 10 methylenes, 12 (4+8carbonyls) quaternary carbons and 28 methine and methyl carbons. The quaternary carbon peak at δ 99.6 ppm was indicative of hydration at C(30).

The 500 MHz ^1H NMR spectrum of **257** in $CDCl_3$ contained a low field shifted signal (δ 9.84 ppm, br s) assignable to the *N*-hydroxy proton. Also apparent were 12 methyl signals and an *N*-Me peak at δ 2.98 ppm. The OH of the lactol was present at δ 6.30 ppm (br s).



257

	δ_C (ppm)	δ_H (ppm)	J_{H-H} (Hz)		δ_C (ppm)	δ_H (ppm)	J_{H-H} (Hz)
Acyl Side chain				**Pip x 2**			
1	175.3			NH		4.39 br d	11.9
2	77.5			α	52.2	5.18 dd	5.8, 1.8
3	99.6				51.7	4.90 dd	7.4, 3.0
4	28.3			β	24.4	2.57 d	13
5	27.3				24.1	2.27 d	12.3
6	32.6			γ	21.5		
7	82.2	3.95 d	10.3		21.2		
8	132.8			δ	47.7	2.60 m, 3.31 d	12.8
9	129.4	5.6 dd	9.1, 1.2		45.7	2.95 m, 3.15 d	13.3
10	38.2	4.05 dq	9.1, 7.0	C=O	174.1		
11	203				169.5		
12	137.5			**N-Me-Leu**			
13	136.8	6.71 dq	7, 1.3	N-Me	29.2	2.98 s	
14	14.7	1.84 dd	6.9, 1.1	α	49.5	6.24 t	7.4
15	25.9			β	36.5		
16	8.2	0.82 t	7.4	γ	24.9		
17	12.1	1.56 d	1.3	δ	22.7	0.92 d	6.5
18	19.4	1.10 d	6.9		22.8	0.94 d	6.5
19	11.4	1.76 t	1.2	C=O	172.2		
20	17.6	0.68 d	6.6	**Thr**			
2-OH		2.91 s		NH		6.16 d	8.4
3-OH		6.30 s		α	56.3	4.50 d	8.4
OH-Leu				β	64.8	4.77 q	6.4
NH		8.22 d	10.7	γ	19.5	1.04 d	6.5
α	54.7	4.89 t	10.7	OH		4.53 br s	
β	78.5	5.41 dd	10.8, 2.2	C=O	170.3		
γ	29.4			**N-OH-Ala**			
δ	14.9	0.70 d	6.9	N-OH		9.84 br s	
	18.9	0.8 d	6.9	α	50.8	5.11 q	7
C=O	170.9			β	13.4	1.47 d	7.2
				C=O	173.6		

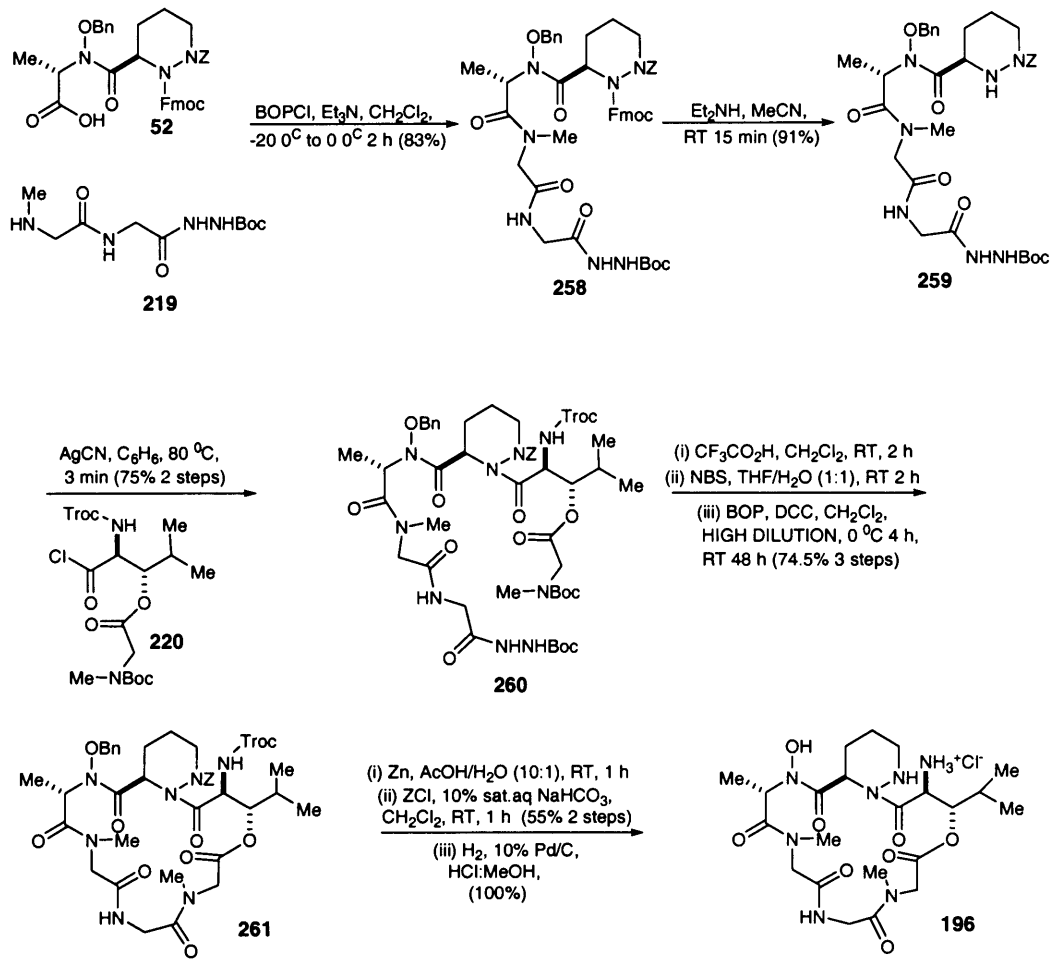
Table 2 125 MHz ^{13}C and 500 MHz ^{1}H NMR data for **257** in $CDCl_3$ at 298K

4.4 Synthesis of an A83586C/Verucopeptin hybrid

As part of our analogue work we synthesised another cyclodepsipeptide core **196** which was identical to verucopeptin, except for the *N*-hydroxy glycine residue, which had been replaced with an *N*-hydroxy-L-alanine residue. The synthesis is shown in Scheme 63. The fragments required were peptide **219** and ester **220** (both common to the synthesis of the verucopeptin cyclodepsipeptide) and peptide **52**, an intermediate used in the synthesis of the GE3 and A83586C cyclodepsipeptides. The synthesis was conducted in exactly the same fashion as our previous syntheses on the cyclodepsipeptide cores.

The BOP-Cl/Et_3N mediated [2+2] coupling between **52** and **219** proceeded very well and was complete after 2 hours at 0 ^{0}C according to TLC analysis; the faster moving product was golden brown when stained with anisaldehyde/H_2SO_4. SiO_2 flash chromatography of the crude reaction mixture using pure EtOAc as eluant afforded tetrapeptide **258** in 83% yield. Fmoc deprotection of **258** with 40 equivalents of Et_2NH in MeCN gave the tetrapeptide **259** in high yield (91%).

The [4+2]–fragment condensation between tetrapeptide **259** and ester **220** proceeded extremely well. Acid chloride **220** was formed by treating the corresponding acid with 35 equivalents of $(COCl)_2$ in dry C_6H_6. Following removal of the excess $(COCl)_2$ *in vacuo*, a solution of 1 equivalent of amine tetrapeptide **260** in dry C_6H_6 and 1.5 equivalents of AgCN were added, and the mixture was heated at 80^{0}C for 2.5 min. Careful control of reaction time is essential in these reactions if one wishes to preserve the Troc group. Therefore, we do not recommend anyone who is repeating this reaction to exceed this reaction duration. Filtration and purification afforded linear hexadepsipeptide **260** as a white solid in 75% overall yield. According to TLC, the reaction was very clean consisting of a major faster moving product that stained golden brown with anisaldehyde/H_2SO_4 stain.



Scheme 63 Synthesis of the A83586C/Verucopeptin hybrid cyclodepsipeptide salt **196**

With just over 2 g of hexadepsipeptide **260** prepared we proceeded to implement our 3 step protocol for inducing deprotection of the glycine and sarcosine residues and bringing about the subsequent macrolactamisation. Treatment of **260** with a large excess of TFA in CH_2Cl_2 at 0 ^{0}C for 2 h, removed the terminal *N*-Boc groups. After azeotropic removal of TFA *in vacuo* with PhMe, the solid residue that was formed was dissolved in a 1:1 mixture of THF/H_2O. To this vigorously stirred solution was added 2 equivalents of NBS in portions over 5 min. After 2 h at RT, the reaction mixture was extracted with EtOAc and concentrated *in vacuo*. This crude residue was then used for the subsequent cyclisation. Initially, for the macrolactamisation, we used our standard high dilution HATU/NEM conditions (10 equiv. of HATU, 13.5 equiv. of NEM, at 0.00043 M) and we obtained the macrolactam **261** as a single product according to TLC in 51% overall yield for the 3

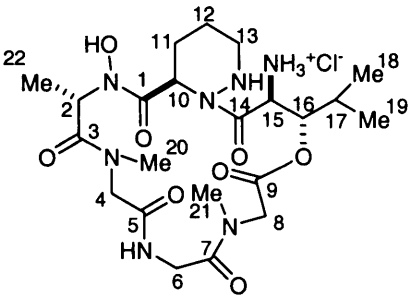
steps. We also attempted a macrolactamisation with the BOP reagent and DMAP. These conditions had been used in the macrolactamisation of 4-*epi*-A83586C.[115] In our case there were no chiral centres at risk in the cyclisation step and the problem of epimerisation was not present. Hence to a solution of 10 equivalents of BOP reagent and 25 equivalents of DMAP in dry CH_2Cl_2 (c = 0.00086 M) at 0 ^{0}C, we added a solution of the crude linear hexadepsipeptide in dry CH_2Cl_2 (c = 0.0013 M). The addition was conducted over 4 hours and the reaction was allowed to stir at RT for 48 h. The overall yield of **261** for the three steps was an excellent 74.5%, the highest we have ever recorded in such a cyclisation.

To complete the synthesis of the target cyclodepsipeptide salt **196**, **261** was subjected to an *N*-Troc to *N*-Z protecting group interchange and a global deprotection by hydrogenating in the presence of 1 equivalent of methanolic HCl and 10% wet Pd/C. Filtration and concentration *in vacuo* afforded the cyclodepsipeptide salt **196** as a white solid in 55% yield for the three steps.

The structure of **196** was confirmed by its FAB HRMS which contained an $(M+H)^+$ peak at *m/e* 514.2600 (Calcd. for: $C_{21}H_{36}O_8N_7$ $(M+H)^+$: 514.2625). Additional evidence to support the assigned structure was provided by the room temperature 500 MHz ^{1}H NMR and 125 MHz ^{13}C NMR spectra of **196** in CD_3OD, which clearly showed that it was a single compound and existed as one confomer. The spectra were virtually identical to those of the verucopeptin cyclodepsipeptide salt **195** and hence we were able to fully assign **196** by comparisons. The assignments are shown in Table 3.

The 125 MHz ^{13}C NMR spectrum of **196** contains 22 carbon signals including six amide/ester carbonyl carbons (δ (ppm) 173.9, 172.5, 172.4, 170.5, 170.1, 169.4). ^{13}C DEPT analysis revealed that 6 methylenes, 6 quaternary carbons and 10 methine and methyl carbons were present in the final structure.

The 500 MHz ^{1}H NMR spectrum of **196** was virtually identical to that of the verucopeptin cyclodepsipeptide salt **195**. The points of difference were the Me of the *N*-hydroxy-alanine residue which resonated at δ 1.33 ppm (d, J = 6.5 Hz) and the corresponding α proton appearing at δ 5.37 ppm (q, J = 6.3 Hz).



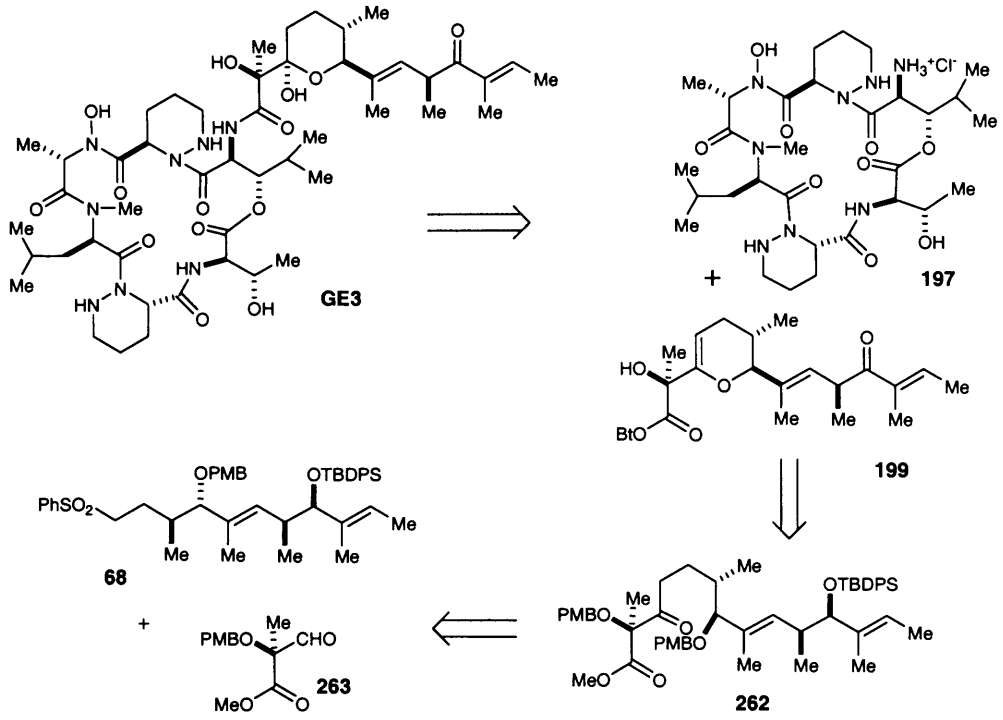
196

Position(s)	125MHZ ^{13}C NMR δ (ppm)	500MHz ^{1}HNMR δ (ppm)
1, 3, 5, 7, 9, 14	173.9, 172.5, 172.4, 170.5, 170.1, 169.4	
		5.37 (q, 6.3 Hz, 1H)
2, 4, 6, 8	54.4, 42.9, 52.8, 53.3	5.08 (d, 17.9 Hz, 1H) 3.76 (d, 17.6 Hz, 1H) 4.47 (d, 17.4 Hz, 1H) 4.04 (d, 17.2 Hz, 1H) 3.92 (d, 4.5 Hz, 1H) 3.89 (d, 4.2 Hz, 1H)
10	51.5	5.08 (1H)
11	24.2	2.1 (d, 13.7 Hz, 1H) 2.0 (m, 1H)
12	21.9	1.64 (d, 13.7 Hz, 1H) 1.54 (m, 1H)
13	47.7	3.12 (m, 1H) 2.72 (m, 1H)
15	50.6	5.29 (br d, 2.7 Hz, 1H)
16	78.6	5.08 (br d, 9.8Hz, 1H)
17	30.6	1.84 (m, 1H)
18,19	19.4, 19.4	1.17 (d, 6.5 Hz, 3H) 0.92 (d, 6.5 Hz, 3H)
20,21	37.5, 35.1	3.15 (s, 3H) 2.87 (s, 3H)
22	14.9	1.33 (d, 6.5 Hz, 3H)

Table 3 ^{13}C and ^{1}H NMR data for **196** in CD_3OD at 298K

4.5 Attempts Toward the Synthesis of the GE3 Pyran

With a successful asymmetric synthesis of the A83586C/GE3 hybrid **257** complete, we now endeavored to synthesise the pyran sector of GE3 **199** with a view to achieving the first asymmetric total synthesis of GE3. Our plan of action is depicted in Scheme 64.



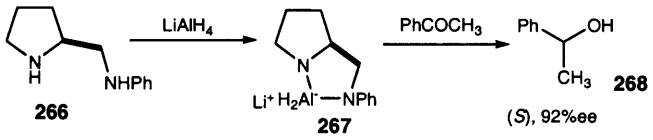
Scheme 64 Retrosynthetic analysis of the GE3 acyl side-chain **199**

Our approach to GE3 was based upon an effective union of the fully elaborated activated ester **199** with the already synthesised GE3 cyclodepsipeptide salt **197**. It was envisioned that, as in the synthesis of A83586C, the resulting glycal would be capable of being chemoselectively hydrated under mildly acidic conditions to afford the natural product. As in the case of A8356C, we envisioned accessing the activated ester **199** from the δ-hydroxy ketone **262**, which itself would be derived from the known sulfone **68** and the α-aldehydo-ester **263**. By using our own published methodology (Schemes 12, 13, 14) we were able to produce over 50 g of this key intermediate to implement this strategy.

Our attention therefore focused upon forming aldehyde **263**; its tertiary alkyl grouping contains a methyl rather than an ethyl (A83586C). This seemingly subtle difference, the presence of the Me rather than the Et, meant that the required GE3 aldehyde **263** could not be synthesised via the same route developed for the A83586C aldehyde **51**; the reason being (Scheme 65) that the tertiary centre in the A83586C aldehyde **51** is set via a Sharpless AD reaction on the 1, 1-disubstituted alkene **69**; a reaction that performs poorly on the corresponding methallyl derivative **264** one would need to obtain aldehyde **263**.[120]

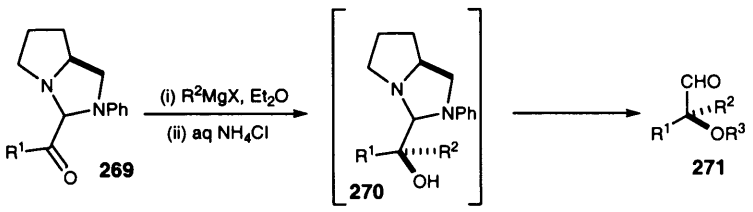
Scheme 65 Sharpless AD reaction on 1, 1-disubstituted alkenes **69** and **264**

A different synthetic strategy was therefore required to enantioselectively set the tertiary centre and access the required α-aldehydo-ester **263**. Our early thoughts centred around the use of Mukaiyama's proline derived keto-aminals (Scheme 66). Mukaiyama had previously used chiral diamines as hydride reducing reagents for the asymmetric reduction of prochiral carbonyl compounds.[121] The chiral inducing agents were prepared from lithium aluminium hydride and (*S*)-2-(*N*-substituted aminomethyl) pyrrolidines **266**, themselves derived easily in four steps from commercially available (*S*)-proline.



Scheme 66 Mukaiyama's use of chiral diamines as hydride reducing reagents

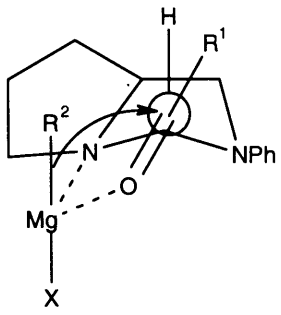
These chiral diamines were further used by Mukaiyama to synthesise chiral keto aminals **269**, which could be conveniently converted to chiral tertiary α-hydroxy aldehydes **271** with high optical yields.[122, 123] The results obtained are shown in Scheme 67.



R^1	R^2MgX	Yield	Ee (%)	Config.
Me	PhMgBr	76	99	R
Me	EtMgBr	43	78	R
Me	CH_2=CHMgBr	44	93	R
Et	PhMgBr	80	100	R
Et	MeMgBr	41	78	S

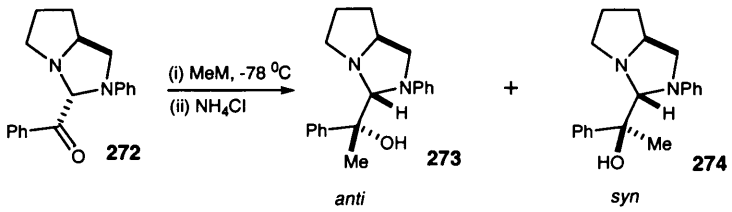
Scheme 67 Mukaiyama's keto-aminals in synthesis of chiral tertiary α-hydroxy aldehydes

The configurations of the tertiary α-hydroxy aldehydes obtained were supported by Mukaiyama's model of enantioselectivity (Scheme 68). The magnesium of the Grignard reagent complexes with the carbonyl oxygen and the nitrogen on the pyrrolidine ring of the keto aminal, which can more strongly complex than the more electron deficient phenyl substituted nitrogen. The resulting rigid magnesium complex allows the Grignard substituent to migrate to the carbonyl carbon from the less hindered side.



Scheme 68 Mukaiyama's model of enantioselectivity

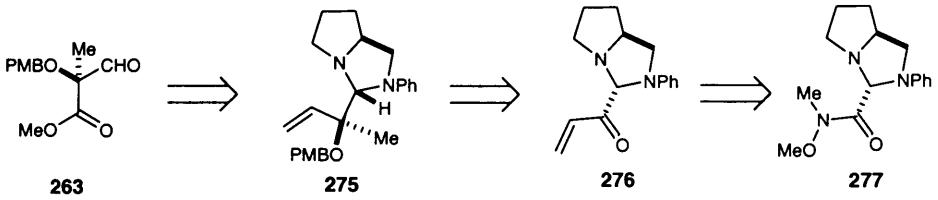
In their studies on the synthesis of optically active δ-hydroxy phosphine oxides, the group of Stuart Warren[124, 125] successfully employed Mukaiyama's proline-derived keto aminals. Primarily they reinvestigated the addition of methyllithium to the phenyl ketone **272** in Et_2O and THF; and addition of MeMgBr was also re-examined. Their results (Scheme 69) were in agreement with Mukaiyama's model of stereoselectivity provided lithium or magnesium counteranions were used in Et_2O. The addition of methyllithium in THF was unselective, however, presumably because the more coordinating solvent interferes with the efficient formation of the chelated intermediate, or possibly changes the steric bulk of the Grignard or RLi reagents.



M	Solvent	*syn : anti*
MgBr	Et_2O	>97:3
Li	Et_2O	95:5
Li	THF	39:61

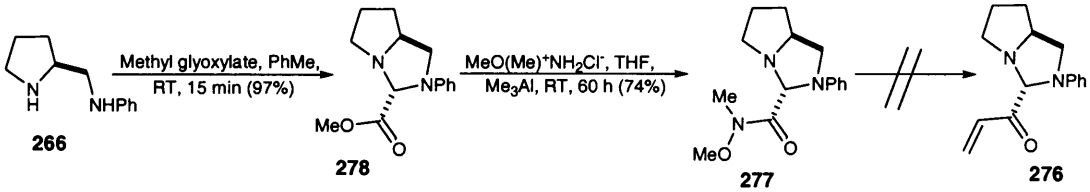
Scheme 69 Warren's use of Mukaiyama's keto aminals

Based on these results we attempted to use Mukaiyama's proline derived keto aminals to set the tertiary centre of our target aldehyde **263**. Our retrosynthetic plan is depicted in Scheme 70. It was thought that vinyl Grignard addition to known Weinreb amide **277** would furnish α, β-unsaturated ketone **276**, which would then be subjected to methyllithium treatment to give **275** (after hydroxy group protection); this would set the required tertiary centre. Aldehyde **263** would then be obtained by acid mediated removal of the keto aminal, oxidation of the ensuing aldehyde to the acid, and subsequent conversion to the methyl ester. Finally oxidative cleavage of the terminal olefin would give aldehyde **263**.



Scheme 70 Retrosynthetic analysis of chiral tertiary α-hydroxy aldehyde **263**

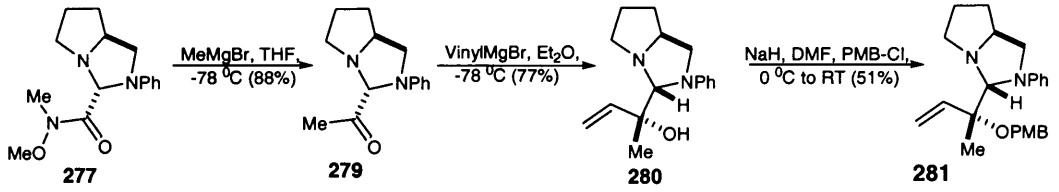
We followed the standard literature procedures[124] to synthesise chiral diamine **266** from (*S*)-proline. This was then converted to the known Weinreb amide **277** (Scheme 71) by first condensing chiral diamine **266** with methyl glyoxylate and converting the ensuing methyl ester **278**, obtained as a single diastereoisomer, to the amide **277**.



Scheme 71 Route towards vinyl ketone **276**

Unfortunately attempts to form vinyl ketone **276**, by the addition of vinyllithium or vinylmagnesium bromide to amide **277** were unsuccessful. Initially we attempted to form the vinyllithium *in situ* by adding 1 equivalent of either *n*-BuLi or *t*-BuLi to 1 equivalent of vinyl bromide in dry THF. A range of such reactions were set up that varied the temperature of vinyllithium

formation. To these, 0.5 equivalents of amide **277** was always added. In every instance, the only product observed was the *n*-butyl or *t*-butyl substituted product. Use of commercially available 1.0 M vinylmagnesium bromide in THF was also unsuccessful. Whilst we believe that the α, β-unsaturated ketone **276** was being formed, we think that it was attacked *in situ*, in a Michael-type 1, 4 addition by the liberated *N, O*-dimethylhydroxylamine. Therefore we altered our strategy slightly (Scheme 72). The known methyl ketone **279** was synthesised by addition of 3 equivalents of a 3.0 M solution of MeMgBr to amide **277**. Methyl ketone **279** was then treated with vinyl magnesium bromide in Et_2O to successfully give tertiary alcohol **280** as a single diastereoisomer by NMR analysis in 77% yield. Protection of the tertiary alcohol as a *p*-methoxybenzyl ether proved problematic because the reaction never went to completion. Nevertheless the *p*-methoxybenzyl ether **281** was isolated in 51% yield. The 500 MHz ^{1}H NMR showed a single diastereoisomer; the PMB group resonated as a pair of doublets at δ 7.10 ppm (d, J = 8.7 Hz) and δ 6.77 ppm (d, J = 8.7 Hz) and a singlet at δ 3.78 ppm corresponding to the OMe; the allyl group appeared at δ 6.05 ppm (dd, J = 17.7 Hz, 10.8 Hz), δ 5.30 ppm (dd, J = 10.8 Hz, 1.5 Hz) and δ 5.30 ppm (dd, J = 17.7Hz, 1.5 Hz); the aminal proton reasonated at δ 4.63 ppm (s) whilst the methyl group appeared at δ 1.47 ppm as a singlet.

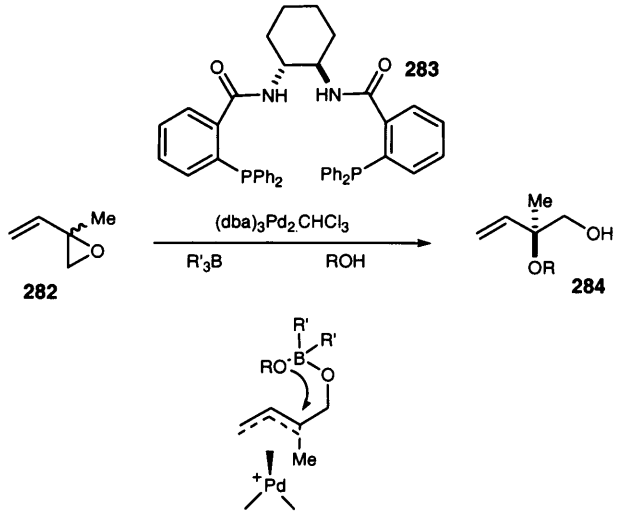


Scheme 72 Synthesis of *p*-methoxybenzyl ether **281**

Purification of the aminal products was slightly problematic via SiO_2 flash chromatography since prolonged exposure of the products on SiO_2 led to some aminal deprotection. Compounds **279** and **280** were formed very cleanly enabling the products to be quickly purified by SiO_2 chromatography.

To access our required aldehyde **263** the terminal vinyl group of **281** needed to be oxidised to the acid and the latter converted to the methyl ester whilst keeping the aminal intact. Unfortunately, after many attempts at implementing this chemistry, the route had to be abandoned after initially showing great promise. Oxidation of the olefin using a variety of methods; AD-mix mediated dihydroxylation followed by $Pb(OAc)_4$ diol cleavage; OsO_4 / $NaIO_4$; and ozonolysis all failed to yield a single product according to TLC analysis. Presumably these conditions also cleave the keto-aminal.

Our efforts at synthesising the desired α-aldehydo ester **263** were eventually rewarded by exploiting a remarkable deracemization reaction developed by Barry Trost.[126, 127, 128] This one-pot reaction involves conversion of a racemic vinyl epoxide to a chiral nonracemic vinylglycidol, which is facilitated by a two-component catalyst system comprising of a palladium source and a trialkylborane. As depicted in Scheme 73 when isoprene monoepoxide **282** was treated with a chiral palladium catalyst (formed *in situ* between $(dba)_3Pd_2.CHCl_3$ and (1*R*, 2*R*)-*N*, *N'*-bis-[2-(diphenylphosphino)benzoyl]-diaminocyclohexane **283**, a π-allylpalladium intermediate resulted which was susceptible to chemo-, regio- and enantioselective nucleophilic addition of an alcohol when a trialkylborane catalyst was present.



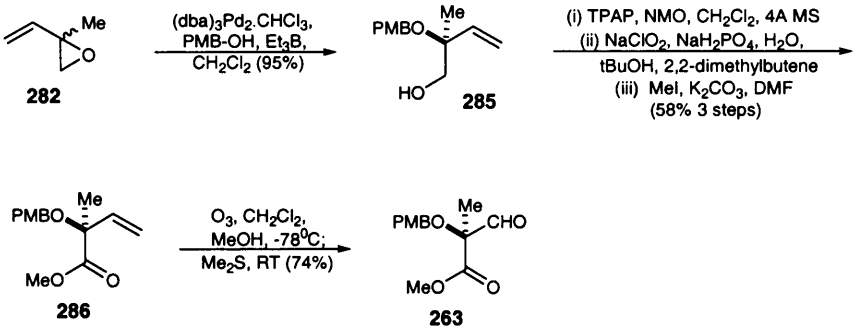
Scheme 73 Trost's deracemization of racemic vinyl epoxide to chiral nonracemic vinylglycidol

Using this chemistry we were able to synthesise aldehyde **263** in five steps (Scheme 74). Application of Trost's procedure on isoprene monoepoxide **282** using *p*-methoxybenzyl alcohol as the nucleophilic species, allowed us to successfully synthesise the known vinyl glycidol **285**. First to a mixture of PMB-OH (1 equiv.), $(dba)_3Pd_2.CHCl_3$ (0.03 equiv.) and (1*R*, 2*R*)-*N*, *N'*-bis-[2-(diphenylphosphino)benzoyl]-diaminocyclohexane **283** (0.01 equiv.) at RT and under inert atmosphere was added dry and degassed CH_2Cl_2. The ensuing purple mixture was left to stir for 15 min at RT to allow formation of the chiral catalyst at which point the reaction colour turned brown/orange. Addition of Et_3B (1 equiv.) and isoprene monoepoxide **282** (1 equiv.) and stirring at RT eventually gave an olive colour on completion (3 h). Removal of the solvent *in vacuo* and SiO_2 flash chromatography afforded vinylglycidol **285** in 95% yield. The 500 MHz ^1H NMR and 125 MHz ^{13}C NMR of **285** was in agreement with that reported by Trost.[126]

Oxidation of **285** to the corresponding acid with PDC (3 to 6 equiv.) was a very slow reaction (4 days), gave rise to a difficult workup, and provided the product in about 50% yield. Therefore we looked for an alternative oxidation method. A two step procedure involving initial oxidation of alcohol **285** to the aldehyde and Pinnick oxidation solved our problems.[129] Aldehyde formation was achieved using TPAP (0.01 equiv.) and NMO (2 equiv.) in dry CH_2Cl_2 containing activated powdered 4A molecular sieves. The crude aldehyde, obtained by filtration and SiO_2 flash chromatography, was subjected to a Pinnick oxidation. This required adding a solution of $NaClO_2$ (3 equiv.), NaH_2PO_4 (3 equiv.) in H_2O to the aldehyde in portions over 15 min. After stirring for 1 h at RT the reaction was complete and the TLC analysis showed a single slower moving major product. Due to the high polarity of the resulting acid and the cleanliness of the crude product by TLC analysis we decided to progress one more step before attempting a purification. Methyl ester **286** was formed by simply treating the acid with K_2CO_3 and MeI in dry DMF. Following SiO_2 flash chromatography methyl ester **286** was produced in an overall 58% yield for the three steps.

All that was required to obtain target aldehyde **263** was ozonolysis of the terminal olefin group of **286**. O_3 was therefore bubbled through a solution of methyl ester **286** in a 20:1 solution of

dry CH_2Cl_2/MeOH at -78 ^{0}C. After 20 min O_2 was bubbled through the reaction mixture and the reaction quenched with 10 equivalents of Me_2S. Purification by SiO_2 flash chromatography afforded aldehyde **263** in 74% yield.

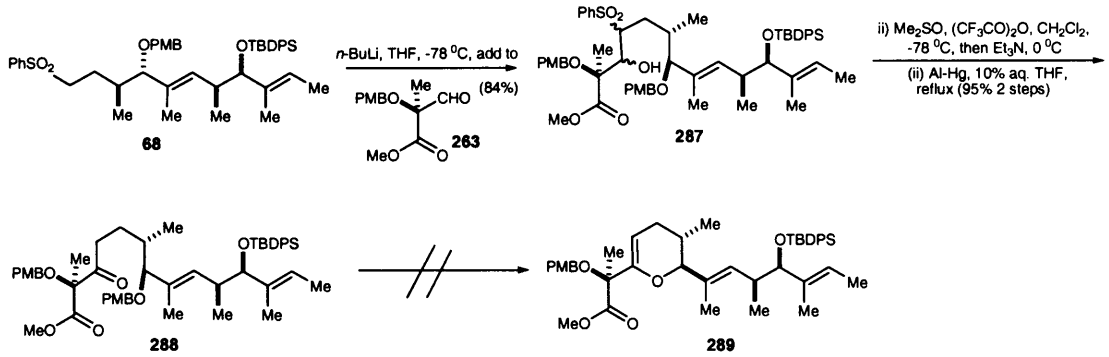


Scheme 74 Synthesis of chiral tertiary α-hydroxy aldehyde **263**

The structure of **263** was confirmed by the room temperature 500 MHz ^{1}H NMR and 125 MHz ^{13}C NMR spectra in $CDCl_3$. The 500 MHz ^{1}H NMR spectrum was assigned as follows; the aldehyde proton resonated as a singlet at δ 9.6 ppm; the PMB ether signals appeared at δ 7.28 ppm (d, J = 8.7 Hz), 6.83 ppm (d, J = 8.7 Hz), 4.48 ppm (2d, AB system, J = 10.4 Hz) and 3.73 ppm (s); the methyl ester resonated at δ 3.72 ppm (s); and the methyl at δ 1.54 ppm (s). The 125 MHz ^{13}C NMR spectrum of **263** contained 11 carbon signals including an aldehyde carbon δ 197.3 ppm, an ester carbon at δ 169.6 ppm and the quaternary carbon α to the aldehyde at δ 84.8 ppm. The ^{13}C DEPT analysis gave 1 methylene, 4 quaternary carbons and 6 methine and methyl carbons.

Aldehyde **263** and phenyl sulfone **68** were coupled as shown in Scheme 75 via formation of the phenylsulfone anion using *n*-BuLi and adding this to aldehyde **263**. Formation of the phenylsulfone anion resulted in a green/yellow colour which dissipated after addition to the aldehyde. The reverse addition was chosen since it was thought that the phenylsulfone anion might attack the methyl ester to give a side product. The ensuing mixture of β-hydroxy sulfones **287** were then oxidised and desulfonylated to yield ketone **288**.

The oxidation of the β-hydroxy sulfones **287** was achieved by Swern oxidation using DMSO (6 equiv.), $(CF_3CO)_2O$ (3quiv.) and Et_3N (10 equiv.). The crude β-keto sulfone was desulfonylated by heating in a refluxing solution of 10:1 THF/H_2O and adding Hg activated Al over 30 min. The latter was formed by dipping strips of Al foil in 2% aq. solution of $HgCl_2$, then washing in MeOH followed by Et_2O. Ketone **288** was isolated after SiO_2 flash chromatography in 96% yield for the two steps; it being obtained as a single diastereoisomer by ^1H NMR.



Scheme 75 Attempt towards the GE3 acyl side-chain

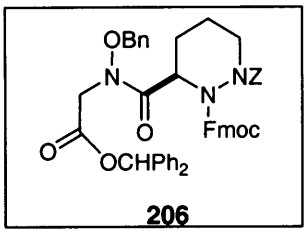
The structure of **288** was confirmed by the 500 MHz ^1H NMR spectrum at 298K in $CDCl_3$. This contained peaks for the two PMB ethers at δ {7.65 ppm (d, J = 7.96 Hz); 7.60 ppm (d, J = 7.96 Hz)}, {6.85 ppm (d, J = 8.83 Hz); 6.75 ppm (d, J = 8.67 Hz)}, {4.45 ppm (d, J = 10.7 Hz); 4.35 ppm (d J = 10.7 Hz)}, 4.15 ppm (d J = 11.4 Hz); 3.80 ppm (d J = 11.4 Hz)}; the olefinic protons appearing as a quartet at δ 4.95 ppm (J = 6.86 Hz, 6.31 Hz) and a doublet at δ 4.80 ppm (J = 10.0 Hz); three singlets at δ (ppm) 3.75, 3.70 and 3.65 corresponding to the two OMe groups and the Me ester. The 125 MHz ^{13}C NMR contained a peak at δ 207.6 ppm for the ketone carbon and δ 170.4 ppm for the ester carbon and the two quaternary olefinic carbons at δ (ppm) 159.2 and 158.8.

We next proceeded to selectively remove the secondary PMB group with DDQ (1.3 equiv.) in CH_2Cl_2. This resulted in a mixture of products by TLC presumed to be δ-hydroxyketone and the hemiketals. As in the synthesis of A83586C efforts were made to dehydrate the mixture in methanol/PPTS to form glycal **289** but none of the required glycal was formed. During the same step of the A83586C synthesis, the tertiary PMB ether was sterically hindered due to the presence of the ethyl group hence the secondary PMB ether can be cleaved selectively. In the case of the GE3 ester the ethyl group is replaced by a methyl group which does not form such a steric hindrance and hence does not allow selective deprotection. We found that once the tertiary PMB ether is cleaved these compounds are not very stable and are amenable to decomposition such as the retro-aldol reaction.

CHAPTER 5 EXPERIMENTAL

Materials and methods

Reactions were carried out under a nitrogen atmosphere with freshly distilled solvents unless otherwise stated. Hexanes refers to the distillate fraction of petroleum spirit that was collected at 40-60 ^{0}C and are distilled prior to use. All solvents were reagent grade. Dichloromethane, toluene, benzene and acetonitrile were distilled from calcium hydride under nitrogen. Diethyl ether and THF were distilled from sodium under nitrogen. All other reagents were used as supplied from the manufacturer. Flash column chromatography was carried out according to Still et al.[130] with Kiesel gel 60 40/60A (220-240 mesh) silica gel. Precoated silica gel plates (250mm) with a fluorescent indicator (E.Merck) were used for analytical thin layer chromatography. The plates were initially examined under UV light and then developed with a sulphuric acid stain [EtOH:H_2SO_4:p-$MeOC_6H_4CHO$ (95:4:1)]. Concentrated *in vacuo* refers to the removal of solvents at <45 ^{0}C on a Buchi rotary evaporator. ^{1}H NMR spectra were acquired at 500 MHz with a Bru¢ker DRX 500 and ^{13}C NMR spectra acquired at 125 MHz with a Brucker DRX 500. Chemical shifts are reported in δ-values relative to tetramethylsilane and all NMR spectra were recorded in deuterated solvents. All infrared spectra were recorded on a Perkin-Elmer 1605 FT-IR spectrophotometer. Optical rotations were measured on an Optical Activity, Polar 2000 automatic polarimeter. High resolution mass spectra were measured at the London school of pharmacy on a V. G. 7070H or V G-ZAB instrument with a Finnigan Incos II data system.



206

To acid **204** (2.8 g, 5.8 mmol) in dry C_6H_6 (10 ml) under N_2 was added $(COCl)_2$ (10 ml, 115 mmol) in one portion at RT, where it was left to stir for 20 min. The reaction mixture was then heated to 50 ^{0}C whereupon it was stirred for 2.5 h. The reaction mixture was concentrated *in vacuo* and the residue coevaporated with C_6H_6 (2 x 8 ml) to remove the excess $(COCl)_2$. The resulting yellow foam was left on the oil pump for 30 min. To this foam was added a solution of amine **205** (2 g, 5.8 mmol) in C_6H_6 (15 ml) at RT under N_2, and with stirring and maintaining the N_2 atmosphere AgCN (1.16 g, 8.6 mmol) was added in one portion. The reaction vessel was fitted with a reflux condenser, covered in aluminium foil and heated to 70 ^{0}C for 45 min under N_2. The reaction mixture was diluted with EtOAc (50 ml), filtered through Celite, and the Celite washed thoroughly with EtOAc. The filtrate was concentrated *in vacuo* and the product purified by SiO_2 flash chromatography (3:1 then 1:1 hexanes/EtOAc) to afford **206** as a white foam (Yield: 4.49 g, 96% 2 steps).

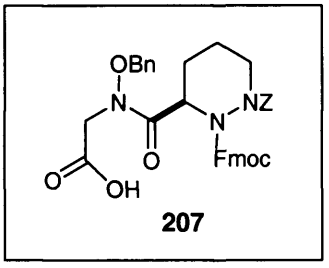
Infra-Red (KBr) (cm^{-1}): 3067 (w), 3033 (w), 2944 (w), 1739 (br s), 1706 (br s), 1494 (w), 1450 (m), 1406 (br m), 1356 (w), 1306 (w), 1283 (w), 1250 (s), 1189 (s), 739 (s), 694 (s)

500 MHz ^{1}H NMR ($CDCl_3$): δ 7.8-7.0 (m, 28 H), 6.87 (2 x s, 1H), 5.55 (br s, 1H), 5.3-5.0 (m, 3H), 4.90 (m, 1H), 4.62 (m, 2H), 4.32 (br s, 2H), 4.13 (m, 1H), 3.72 (m, 1H), 2.89 (m, 1H), 2.20 (br s, 1H), 1.82 (m, 1H), 1.68 (br s, 1H), 1.32 (br s, 1H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 173.0, 172.3, 166.3, 166.2, 155.8, 155.5, 143.8, 143.5, 141.2, 139.7, 139.5, 136.1, 134.0, 129.5, 129.0, 128.4, 128.3, 127.6, 127.5, 125.0, 119.9, 78.1, 68.9, 67.6, 67.5, 50.2, 49.8, 49.3, 49.0, 47.1, 46.9, 45.6, 44.1, 24.9, 24.2, 19.2, 18.5

FAB HRMS: Calcd. for $C_{50}H_{45}O_8N_3Na$ $(M+Na)^+$: 838.3104; Found: 838.3090

Optical Rotation: $[\alpha]_D$ = +7.5 ^{0} (*c* 1.02, CH_2Cl_2)



207

To dipeptide **206** (13 g, 16 mmol) in dry CH_2Cl_2 (170 ml) at 0 ^{0}C under N_2 was added PhOH (2.2 g, 24 mmol) followed by CF_3CO_2H (44 ml, 576 mmol) dropwise over 10 min. The reaction mixture was stirred at 0 ^{0}C for 2 h, concentrated *in vacuo* and coevaporated with PhMe (3 x 30 ml) to remove the excess CF_3CO_2H. The product was purified by SiO_2 flash chromatography (5:1 then 0:1 hexanes/EtOAc) to afford **207** as a white foam (Yield: 9.9 g, 96%).

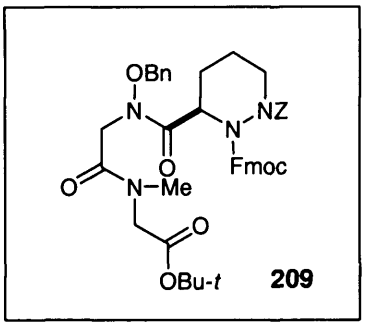
Infra-Red (KBr) (cm^{-1}): 3435 (br w), 3034 (w), 2953 (w), 1707 (br s), 1686 (br s), 1499 (w), 1450 (m), 1412 (br m), 1358 (w), 1312 (w), 1256 (m), 1196 (m), 741 (m)

500 MHz ^{1}H NMR ($CDCl_3$): δ 10.6 (br s, 1H), 8-7 (m, 18 H), 5.62 (br s), 5.4-4.83 (m), 4.71 (m), 4.55 (d, *J* = 17.1 Hz), 4.35-4.27 (br s), 4.27-4.0 (m), 3.91 (br d, *J* = 17.1 Hz), 3.72 (br d, *J* = 17.1 Hz), 2.95 (m), 2.30 (br s, 1H), 2.02 (br s, 1H), 1.79 (br s, 1H), 1.47 (br s, 1H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 172.1, 171.5, 171.0, 155.6, 155.4, 155.0, 143.4, 142.9, 141.1, 136.7, 136.5, 134.3, 134.0, 129.3, 129.1, 128.9, 128.7, 128.4, 128.3, 127.9, 127.5, 127.1, 124.5, 119.9, 78.3, 68.5, 68.0, 67.3, 50.2, 49.7, 49.3, 48.9, 46.7, 46.5, 45.2, 44.0, 23.5, 23.1, 19.1, 18.3

FAB HRMS: Calcd. for $C_{37}H_{35}O_8N_3Na$ $(M+Na)^+$: 672.2322; Found: 672.2300

Optical Rotation: $[\alpha]_D$ = +5.0 ^{0} (*c* 1.25, CH_2Cl_2)



209

To the hydrochloride salt of the *tert*-butyl ester of sarcosine (0.29 g, 1.6 mmol) was added sat. aq. $NaHCO_3$ (10 ml) and Et_2O (15 ml) and the reaction mixture was shaken. The ethereal layer was removed and the aq. layer was further extracted with Et_2O (2x15 ml). The combined Et_2O layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield a viscous colourless oil **208**. To this amine **208** at -20 ^{0}C under N_2 was added a solution of dipeptide **207** (0.8 g, 1.2 mmol) in dry CH_2Cl_2 (15 ml) in one portion, followed by Et_3N (0.34 ml, 2.5 mmol), and BOP-Cl (0.38 g, 1.5 mmol) in one portion the reactants stirred at this temperature for 20 min. The reaction mixture was then warmed to 0 ^{0}C where it was stirred for 3 h. The reaction mixture was diluted with CH_2Cl_2 (50 ml) and washed with 0.5 M aq. HCl (3 x 20 ml), H_2O (3 x 20 ml), 5% aq. $NaHCO_3$ (2 x 20 ml) and brine (20 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by SiO_2 flash chromatography (3:2 then 1:1 hexanes/EtOAc) to furnish **209** as a white foam (Yield: 0.78 g, 82%, 2 steps).

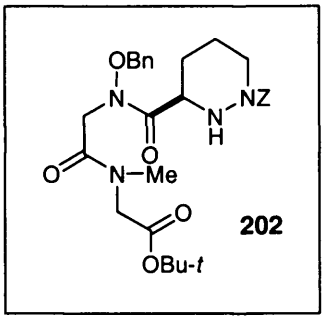
Infra-Red (KBr) (cm^{-1}): 3063(w), 3030(w), 2976(w), 2932(w), 1736(s), 1703(s), 1660(s), 1453(s), 1409(s), 1360(s), 1284(s), 1251(s), 1191(m), 1153(m), 1126(m), 1093(m), 1049(m), 984(w), 940(w), 913(w), 848(w), 815(w), 739(m), 695(m), 619(w), 542(w), 502(w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 8.0-7.0 (m, 18 H), 5.8-3.4 (broad signals, 13H), 2.91 (2 x br s, 3H, 1H), 2.33 (br s, 1H), 2.11 (br s, 1H), 1.81 (br s, 1H), 1.40 (br s superimposed on s, 9H, 1H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 168.1, 167.0, 156.3, 156.2, 155.3, 143.4, 143.2, 141.0, 138.1, 129.8, 128.3, 128.0, 127.9, 127.3, 126.1, 119.2, 83.6, 82.1, 69.2, 68.6, 68.4, 51.3, 50.0, 49.3, 48.4, 48.3, 46.0, 44.1, 42.0, 35.1, 30.9, 29.0, 25.3, 24.9, 19.5, 19.0

FAB HRMS: Calcd. for $C_{44}H_{48}O_9N_4Na$ $(M+Na)^+$: 799.3319; Found: 799.3303

Optical Rotation: $[\alpha]_D$ = $+15.7^0$ (*c* 0.64, CH_2Cl_2)



202

To tripeptide **209** (0.35 g, 0.451 mmol) in MeCN (1.86 ml) at RT under N_2 was added Et_2NH (1.86 ml) in one portion. The reaction mixture was left to stir for 1 h after which it was concentrated *in vacuo* and the residue was purified by SiO_2 flash chromatography (10:1 then 0:1 hexanes/EtOAc) to afford **202** as a very viscous colourless oil (Yield: 0.24 g, 95%).

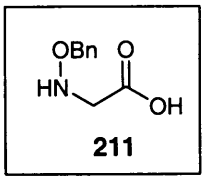
Infra-Red (KBr) (cm^{-1}): 3493 (w), 3262 (w), 3054 (w), 3008 (w), 2974 (w), 2927 (w), 1737 (m), 1685 (s), 1662 (s), 1494 (m), 1448 (m), 1396 (m), 1367 (m), 1258 (m), 1229 (m), 1160 (m), 1119 (m), 1033 (w), 986 (w), 946 (w), 917 (w), 848 (w), 750 (m), 704 (m)

500 MHz ^{1}H NMR ($CDCl_3$): δ 7.32 (m, 10H), 5.28-5.03 (br s, 2H), 5.03-4.78 (br s, 2H), 4.71-4.39 (m, 1H), 4.30-4.04 (d, *J* = 17.9 Hz, 2H), 4.04-3.6 (m, 4H), 2.92 (2 x s, 3H, 1H), 2.06 (br s, 1H), 1.76 (br s, 1H), 1.61 (br s, 1H), 1.40 (br s superimposed on br s, 9H, 1H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 174.2, 167.7, 167.4, 166.4, 166.2, 155.6, 136.6, 134.5, 129.2, 128.7, 128.5, 128.3, 127.8, 82.8, 81.8, 77.7, 67.2, 56.3, 51.7, 50.1, 49.4, 45.1, 35.3, 27.9, 27.2, 23.0

FAB HRMS: Calcd. for $C_{29}H_{38}O_7N_4Na$ $(M+Na)^+$: 577.2638; Found: 577.2660

Optical Rotation: $[\alpha]_D$ = $+3.5^0$ (*c* 0.28, CH_2Cl_2)

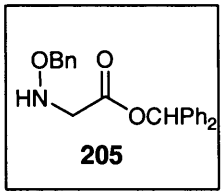


211

To the hydrochloride salt of *O*-benzylhydroxylamine (10 g, 62.6 mmol) in dry MeOH (50 ml) was added dry Et_3N (8.73 ml, 62.6 mmol) in one portion and the reaction mixture was stirred for 10 min at RT. The reaction mixture was concentrated *in vacuo*, H_2O (50 ml) added and extracted with Et_2O (3x100 ml). The combined Et_2O layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield a colourless oil. To this oil was added EtOH (30 ml) and bromoacetic acid **210** (2.17 g, 15.7 mmol) and the reaction mixture stirred for 2 weeks at RT under N_2. The solvents were removed *in vacuo* and to the resulting white solid residue, 10% aq. Na_2CO_3 (30 ml) was added, and this extracted with Et_2O (3 x 15 ml) to remove the excess *O*-benzyl-hydroxylamine. The aqueous layer was acidified to pH 3 with cautious addition of concentrated HCl, upon which, a white precipitate started to form. The solution was left to stand at 0 ^{0}C for 30 min before the solid was filtered off. The product **211** was washed with H_2O and recrystallised from EtOH/H_2O (Yield: 1.9 g, 67% 2 steps).

500 MHz ^{1}H NMR ($CDCl_3$): δ 7.30 (m, 5H), 4.71 (s, 2H), 3.62 (s, 2H)

FAB HRMS: Calcd. for $C_9H_{11}O_3NNa$ $(M+Na)^+$: 204.0637; Found: 204.0644



205

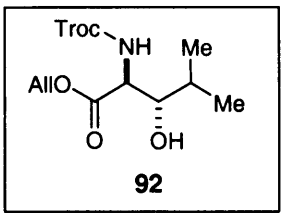
To the *N*-benzyloxyglycine **211** (0.8 g, 4.4 mmol) in acetone (5.5 ml) was added in portions via a Pasteur pipette a solution of diphenyldiazomethane (1.11 g, 5.7 mmol) in acetone (5.5 ml). The reaction mixture was left to stir at RT for 24 h, after which it was concentrated *in vacuo* below 30 ^{0}C. The product was purified by SiO_2 flash chromatography (11:1 then 8:1 hexanes/Et_2O), to yield **205** as a yellow oil (Yield: 1.2 g, 78%).

Infra-Red (neat) (cm^{-1}): 3275 (w), 3088 (w), 3063 (m), 3030 (m), 2918 (w), 2864 (w), 1744 (s), 1601 (w), 1585 (w), 1495 (m), 1454 (m), 1398 (w), 1366 (m), 1310 (w), 1277 (m), 1180 (br s), 968 (br m), 745 (br s), 698 (s)

500 MHz ^{1}H NMR ($CDCl_3$): δ 7.25 (br s, 15H), 6.90 (br s, 1H), 6.05 (br s, 1H), 4.65 (br s, 2H), 3.64 (br s, 2H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 170.1, 139.7, 137.6, 128.5, 128.34, 128.32, 128.0, 127.8, 127.2, 77.6, 76.1, 53.8

FAB HRMS: Calcd. for $C_{22}H_{22}O_3N$ $(M+H)^+$: 348.1600; Found: 348.1585



92

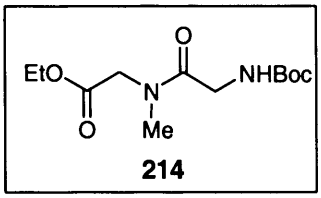
To a solution of (2*S*, 3*S*)-3-hydroxyleucine **39** (2 g, 13.6 mmol) in THF (25 ml) at 0 ^{0}C was added 2M aq. NaOH (6.8 ml, 27.2 mmol). Then with stirring and at 0 ^{0}C TrocCl (1.87 ml, 13.6 mmol) and 2M aq. NaOH (6.8 ml, 27.2 mmol) were added simultaneously to the reaction mixture over 8 min, and allowed to stir at this temperature for 48 h. The reaction mixture was diluted with Et_2O (150 ml) and the aqueous layer extracted and acidified to pH 2 by cautious addition of concentrated HCl. The aqueous layer was then extracted with EtOAc (4 x 100 ml), and the combined EtOAc layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield an off white foam. To the above foam (3.5 g, 10.9 mmol) in dry DMF (35 ml) at RT under N_2 was added solid $NaHCO_3$ (3.65 g, 43.4 mmol) followed by AllBr (5.82 ml, 67.3 mmol). The reactants were stirred at RT for 2 days, then diluted with EtOAc (200 ml), and washed with H_2O (4 x 150 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (6:1 then 3:1 hexanes/EtOAc) to afford **92** as a colourless oil (Yield: 3.53 g, 72% 2 steps).

400 MHz ^{1}H NMR ($CDCl_3$): δ 6.05 (br d, *J* = 8.5 Hz, 1H), 5.89 (m, 1H), 5.33 (dd, *J* = 17.1, 1.3 Hz, 1H), 5.27 (dd, *J* = 10.4, 1.3 Hz, 1H), 4.71 (2 x d, *J* = 12 Hz, 2H), 4.65 (br d, *J* = 5.1 Hz, 2H), 4.51 (dd, *J* = 8.5, 3.8 Hz, 1H), 3.50 (ddd, *J* = 8.4, 7.5, 3.8 Hz, 1H), 2.49 (br d, *J* = 7.5 Hz, 1H), 1.78 (m, 1H), 0.99 (d, *J* = 6.6 Hz, 6H)

100 MHz ^{13}C NMR ($CDCl_3$): δ 170.6, 154.7, 131.6, 119.6, 95.7, 79.0, 75.2, 66.7, 57.0, 31.5, 19.5, 19.9

FAB HRMS: Calcd. for: $C_{12}H_{19}O_5NCl_3$ $(M+H)^+$: 362.0329; Found: 362.0318

Optical Rotation: $[\alpha]_D$ = -10.5 ^{0} (*c* 0.38, CH_2Cl_2)



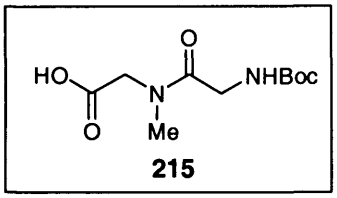
214

To a solution of *N*-Boc-glycine **212** (3.43 g, 19.5 mmol) and the hydrochloride salt of sarcosine ethyl ester **213** (3 g, 19.5 mmol) in CH_2Cl_2 (50 ml) at RT and under N_2 was added sequentially Et_3N (2.8 ml, 20 mmol), DCC (4.06 g, 19.7 mmol) and DMAP (0.36 g, 2.9 mmol). The reactants were allowed to stir for 24 h and then the white solid that had formed during the course of the reaction was filtered off and the filtrate concentrated *in vacuo*. The crude residue was purified by SiO_2 flash chromatography (5:1 then 3:2 hexanes/EtOAc) to afford **214** as a white solid (Yield: 4.27 g, 80%).

500 MHz ^{1}H NMR ($CDCl_3$): δ 5.35 (br s, 1H), 4.10 (q, *J* = 7.2 Hz, 2H), 4.05 and 3.86 (2 x s, 2H), 3.92 and 3.79 (2 x br s, 2H), 2.94 (2 x s, 3H), 1.33 (s, 9H), 1.17 (t, *J* = 7.2 Hz, 3H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 169.1, 168.8, 168.6, 168.1, 155.6, 79.3, 61.6, 61.1, 50.2, 49.4, 42.0, 41.9, 35.0, 34.8, 28.1, 13.9

FAB HRMS: Calcd. for: $C_{12}H_{22}O_5N_2Na$ $(M+Na)^+$: 297.1426; Found: 297.1420



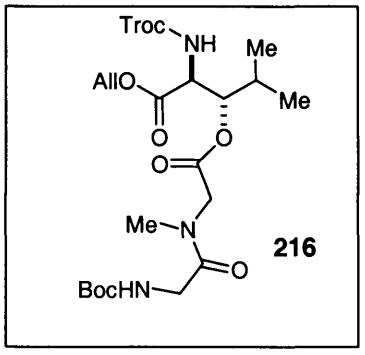
215

To a solution of dipeptide **214** (3.5 g, 12.8 mmol) in THF: CH_3OH: H_2O (3:1:1) (55 ml) at RT was added LiOH monohydrate (1.63 g, 38.9 mmol) and the reactants allowed to stir for 2 h. The reaction mixture was poured into aq. HCl (3 M, 30 ml) and then extracted with EtOAc (3 x 50 ml). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield **215** as a viscous colourless oil that was sufficiently pure for next stage (Yield: 3.14 g, 100%).

500 MHz ^{1}H NMR ($CDCl_3$): δ 10.85 (br s, 1H), 5.64 (2 x br s, 1H), 4.1-3.89 (3 x s, 4H), 2.98 (2 x s, 3H), 1.34 (s, 9H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 171.6, 169.9, 169.5, 156.1, 79.9, 50.2, 49.4, 42.1, 42.0, 35.3, 35.1, 28.2

FAB HRMS: Calcd. for: $C_{10}H_{18}O_5N_2Na$ $(M+Na)^+$: 269.1113; Found: 269.1123



216

To a solution of dipeptide **215** (0.34 g, 1.38 mmol) and Allyl- and Troc- protected 3-hydroxyleucine **92** (0.5 g, 1.38 mmol) in CH_2Cl_2 (7 ml) at RT and under N_2 was added DCC (0.301 g, 1.46 mmol) and DMAP (0.168 g, 1.38 mmol). The reaction was allowed to stir for 24 h and the

white solid that had formed during the course of the reaction was filtered off and the filtrate concentrated *in vacuo*. The crude residue was purified by SiO_2 flash chromatography (4:1 then 2:1 hexanes/EtOAc) to afford **216** as a very viscous colourless oil (Yield: 0.59 g, 73%).

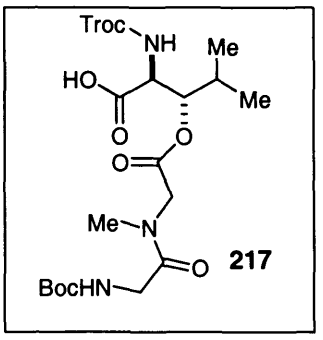
Infra-Red (KBr) (cm^{-1}): 3424 (m), 3308 (m), 3054 (w), 2962 (m), 2881 (m), 1754 (s), 1667 (s), 1488 (s), 1413 (m), 1390 (m), 1367 (m), 1252 (s), 1171 (s), 1119 (m), 1096 (m), 1050 (m), 986 (m), 952 (m), 871 (w), 819 (m), 767 (w), 727 (m), 565 (m)

500 MHz ^{1}H NMR ($CDCl_3$): δ 6.3 (br d, *J* = 8.9 Hz, 1H), 5.84 (m, 1H), 5.43 (br s, 1H), 5.25 (dd, *J* = 17.1, 1.2 Hz, 1H), 5.2 (dd, *J* = 10, 1.2 Hz, 1H), 4.9 (dd, *J* = 8.7, 3.2 Hz, 1H), 4.66 (br s, 2H), 4.63 (dd, *J* = 8.9, 3.2 Hz, 1H), 4.55 (d, *J* = 5.8 Hz, 2H), 4.12 (d, *J* = 17.3 Hz, 1H), 3.95 (br s, 2H), 3.82 (d, *J* = 17.3 Hz, 1H), 2.99 (s, 3H), 1.98 (m, 1H), 1.35 (s, 9H), 1.1-0.8 (2 x d, *J* = 6.7 Hz, 6H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 169.8, 168.5, 168.4, 155.6, 154.1, 131.0, 119.1, 95.2, 79.8, 79.4, 74.4, 66.2, 55.1, 50.3, 42.2, 35.6, 29.1, 28.2, 18.8, 18.5

FAB HRMS: Calcd. for: $C_{22}H_{34}O_9N_3Cl_3Na$ $(M+Na)^+$: 612.1258; Found: 612.1234

Optical Rotation: $[\alpha]_D = -7.3^0$ (*c* 0.72, CH_2Cl_2)



217

To a solution of tripeptide **216** (1 g, 1.69 mmol) in dry THF (20 ml) at RT under N_2 was added morpholine (1.5 ml, 1.74 mmol) followed by $(Ph_3P)_4Pd$ (0.2 g, 0.174 mmol) and the reactants were allowed to stir for 20 min. Et_2O (200 ml) was added and the reaction mixture was washed with aq. $KHSO_4$ (1 M, 3 x 100 ml) and brine (100 ml). The organic layer was dried over $MgSO_4$,

filtered and concentrated *in vacuo*. The crude residue was purified by SiO_2 flash chromatography (2:1 then 3:2 hexanes/EtOAc) to afford **217** as a white foam (Yield: 0.68 g, 73%).

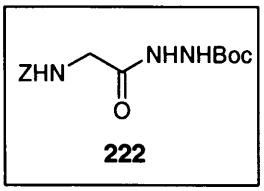
Infra-Red (KBr) (cm^{-1}): 3424 (m), 3331 (m), 2974 (m), 2939 (w), 1737 (s), 1656 (m), 1512 (m), 1413 (w), 1390 (w), 1361 (w), 1252 (m), 1200 (m), 1165 (m), 1119 (w), 1096 (w), 1050 (w), 813 (w), 732 (w), 692 (w), 571 (w), 536 (w)

500 MHz ^1H NMR ($CDCl_3$): δ 10.8 (br s, 1H), 6.62 and 6.35 (br d, J = 8.7 Hz, 1H), 5.81 and 5.62 (br s, 1H), 4.98 (m, 1H), 4.75-4.5 (2xd, J = 11.9 Hz, br d, J = 5.8 Hz, 2H, m, 1H), 4.3-3.8 (complex m, 4H), 3.05 (2 x s, 3H), 2.15 (m, 1H), 1.35 (s, 9H), 1.1-0.7 (2 x d, J = 6.7 Hz, 6H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 170.8, 170.5, 170.2, 170.0, 168.3, 168.1, 156.6, 156.5, 154.1, 153.9, 95.2, 80.9, 80.3, 74.6, 54.9, 50.3, 42.1, 35.7, 35.2, 29.2, 29.0, 28.2, 18.9, 18.6

FAB HRMS: Calcd. for: $C_{19}H_{30}O_9N_3Cl_3Na$ $(M+Na)^+$: 572.0945; Found: 572.0928

Optical Rotation: $[\alpha]_D$ = +11.8 ^{0} (*c* 0.48, CH_2Cl_2)



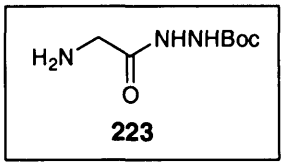
222

To Cbz-glycine **221** (10 g, 48 mmol) in dry THF (150 ml) at 0 ^{0}C under N_2, was added in sequence DCC (19.8 g, 96 mmol), $BocNHNH_2$ (12.7 g, 96 mmol) and HOBt: H_2O (12.96 g, 96 mmol). The reaction mixture was left to stir at 0 ^{0}C for 1 h and then warmed to RT for 24 h, after which Et_2O (300 ml) was added and the solid residue filtered. The filtrate was washed with 1 M aq. HCl (2 x 100 ml), 5% aq. $NaHCO_3$ (2 x 100 ml), and brine (2 x 100 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (4:1, 1:1, 1:2 hexanes/EtOAc) to afford **222** as a white foam (Yield: 15.1 g, 98%).

500 MHz ^{1}H NMR ($CDCl_3$): δ 8.7 (br s, 1H), 7.31 (m, 5H), 6.96 (br s, 1H), 6.0 (br s, 1H), 5.05 (s, 2H), 4.9 (br s, 2H), 1.4 (s, 9H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 169.5, 156.9, 155.6, 136.0, 128.4, 128.1, 128.0, 81.8, 67.2, 42.9, 28.1

FAB HRMS: Calcd. for: $C_{15}H_{21}O_5N_3Na$ $(M+Na)^+$: 346.1379; Found: 346.1390



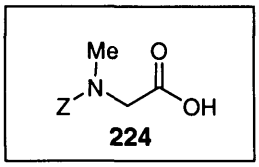
223

To the *tert*-butyl carbazate amide of Cbz- Glycine **222** (15.1 g, 47 mmol) in MeOH (80 ml) at RT was added 20% $Pd(OH)_2$ on carbon (3.23 g, 4.7 mmol). The system was purged with H_2 and stirred vigorously at RT for 8 h. The reaction mixture was diluted with MeOH (200 ml) and filtered through a pad of Celite. The filter pad was washed well with MeOH, and the filtrate concentrated *in vacuo* to yield **223** as a white solid (Yield: 8.5 g, 96%).

500 MHz ^{1}H NMR ($CDCl_3$): δ 4.7 (br s), 3.3 (br s, 2H), 1.35 (br s, 9H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 175.8, 158.6, 84.6, 43.5, 29.0

FAB HRMS: Calcd. for: $C_7H_{16}O_3N_3$ $(M+Na)^+$: 190.1192; Found: 190.1197



224

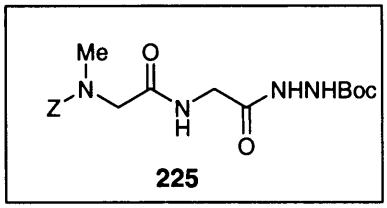
To sarcosine (10.0 g, 112 mmol) in THF (120 ml) and 2 M aq. NaOH (56 ml, 112 mmol) at 0 ^{0}C was added benzyl chloroformate (17.6 ml, 123 mmol) and 2 M aq. NaOH (56 ml, 112 mmol) simultaneously and dropwise over 15 min. The reactants were allowed to stir at 0 ^{0}C for 24 h. The

reaction mixture was diluted with Et_2O (200 ml) and the aqueous layer was extracted. The aqueous layer was acidified to pH 2 with concentrated HCl and extracted with Et_2O (4 x 100 ml). The combined ether layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield **224** as a clear oil that eventually solidified to a white solid (Yield: 24.6 g, 98%).

500 MHz ^{1}H NMR ($CDCl_3$): δ 10.78 (br s, 1H), 7.3 (m, 5H), 5.15 (2 x s, 2H), 4.05 (2 x s, 2H), 2.98 (s, 3H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 174.5, 156.9, 156.1, 136.2, 128.4, 127.8, 127.0, 67.7, 67.6, 50.4, 50.1, 35.9, 35.3

FAB HRMS: Calcd. for: $C_{11}H_{14}O_4N$ $(M+Na)^+$: 224.0923; Found: 224.0925



225

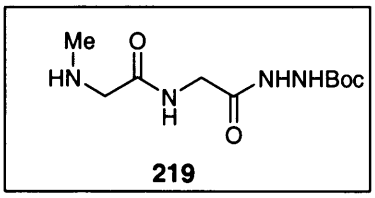
To *N*-Z-sarcosine **224** (9.03 g, 40 mmol) and the *t*-butyl carbazate amide of glycine **223** (7.65 g, 40 mmol) in dry CH_2Cl_2 (150 ml), under N_2 and at RT, was added DCC (8.7 g, 42 mmol) and DMAP (0.98 g, 8 mmol). The reaction mixture was stirred for 24 h after which CH_2Cl_2 (150 ml) was added and the solid residue present removed via filtration. The filtrate was concentrated *in vacuo* and the product purified by SiO_2 flash chromatography (1:3, 0:1 hexanes/EtOAc) to afford **225** as a white solid (Yield: 11.6 g, 73%).

Infra-Red (KBr) (cm^{-1}): 3296 (br s), 3034 (w), 2980 (m), 2936 (w), 1686 (br, s), 1541 (br s), 1487 (br s), 1456 (w), 1406 (m), 1367 (m), 1244 (br s), 1157 (s)

500 MHz ^{1}H NMR ($CDCl_3$): δ 8.85 (br s), 8.65 (br s), 7.85 (br s), 7.32 (m, 5H), 6.91 (br s, 1H), 5.10 (s, 2H), 3.92 (br s, 4H), 3.0 (br s, 3H), 1.41 (s, 9H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 170.0, 169.1, 157.2, 155.6, 136.2, 128.4, 128.0, 127.8, 81.6, 67.7, 52.7, 52.2, 41.4, 35.9, 35.6, 28.1

FAB HRMS: Calcd. for: $C_{18}H_{26}O_6N_4Na$ $(M+Na)^+$: 417.1750; Found: 417.1765



219

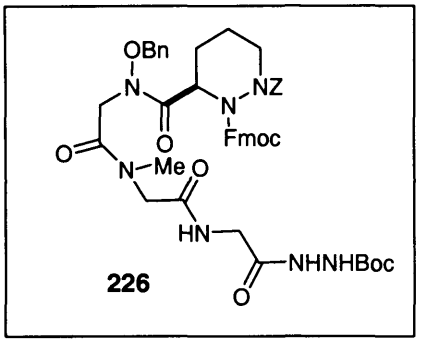
To dipeptide **225** (11 g, 28 mmol) in MeOH (100 ml) at RT was added 20% $Pd(OH)_2$ on carbon (1 g, 1.4 mmol). The system was purged with H_2 and stirred vigorously at RT for 8 h. The reaction mixture was diluted with MeOH (200 ml) and filtered through a pad of Celite. The filter pad was washed well with MeOH, and the filtrate concentrated *in vacuo* to yield **219** as a white solid (Yield: 7.25 g, 100%).

Infra-Red (KBr) (cm^{-1}): 3314 (br s), 2980 (m), 2936 (m), 2802 (w), 1670 (br s), 1533 (br s), 1458 (w), 1414 (w), 1394 (w), 1369 (m), 1248 (br s), 1161 (s)

500 MHz ^{1}H NMR ($CDCl_3$): δ 8.0 (br s), 7.78 (br s), 3.96 (br s, 2H), 3.22 (s, 2H), 2.37 (s, 3H), 1.39 (s, 9H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 172.8, 169.3, 155.8, 81.4, 54.2, 41.2, 36.4, 28.1

FAB HRMS: Calcd. for: $C_{10}H_{21}O_4N_4$ $(M+Na)^+$: 261.1563; Found: 261.1550



226

To dipeptide **219** (3.72 g, 14.3 mmol) at RT under N_2 was added a solution of dipeptide **207** (8.44 g, 13 mmol) in dry CH_2Cl_2 (165 ml). The reaction mixture was cooled to -20 ^{0}C and Et_3N (3.6 ml, 20.6 mmol) followed by BOP-Cl (3.97 g, 15.6 mmol) were added. This was allowed to stir at -20 ^{0}C for 20 min and then stirred at 0 ^{0}C for 5 h. The reaction mixture was diluted with CH_2Cl_2 (200 ml), and washed with 0.5 M aq. HCl (3 x 70 ml), H_2O (2 x 70 ml), 5% aq. $NaHCO_3$ (2 x 70 ml) and brine (70 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (1:3, 0:1 hexanes/EtOAc) to afford **226** as a white foam (Yield: 9.5 g, 82%).

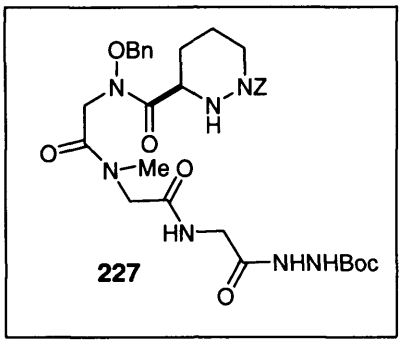
Infra-Red (KBr) (cm^{-1}): 3303 (br s), 3034 (w), 2944 (w), 1699 (br s), 1536 (m), 1497 (s), 1452 (s), 1413 (s), 1368 (s), 1250 (s), 1194 (m), 1160 (s)

500 MHz ^{1}H NMR ($CDCl_3$): δ 8.95-8.03 (br m), 7.8-6.95 (br m), 5.55 (br m), 5.3-5.0 (br m), 4.9-5.0 (br m), 4.85-4.5 (br m), 4.35 (br m), 4.25-3.8 (br m), 2.9 (br m), 2.55 (br m), 2.25 (br m), 2.05 (br m), 1.8 (br m), 1.4 (br s)

125 MHz ^{13}C NMR ($CDCl_3$): δ 172.6, 169.3, 166.9, 155.7, 143.5, 143.2, 141.0, 136.4, 136.3, 129.7, 129.0, 128.7, 128.3, 128.0, 127.9, 127.0, 125.0, 119.7, 81.0, 68.8, 67.8, 67.5, 52.1, 51.9, 49.9, 49.1, 47.0, 46.9, 45.3, 44.0, 41.2, 41.1, 36.0, 35.1, 28.0, 24.2, 23.5, 19.5, 19.0

FAB HRMS: Calcd. for: $C_{47}H_{53}O_{11}N_7Na$ $(M+Na)^+$: 914.3701; Found: 914.3720

Optical Rotation: $[\alpha]_D$ = -60.8 ^{0} (*c* 0.3, CH_2Cl_2)



227

To tetrapeptide **226** (9.8 g, 11 mmol) in MeCN (92 ml) was added Et_2NH (46 ml, 440 mmol) and stirred at RT for 20 min. The reaction mixture was diluted with EtOAc and concentrated *in vacuo* and the product purified by SiO_2 flash chromatography (1:1, 0:1 hexanes/EtOAc then 10:1 EtOAc/MeOH) to afford **227** as a white solid (Yield: 6.38 g, 87%).

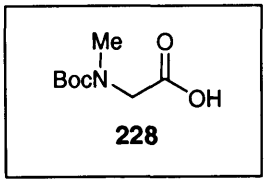
Infra-Red (KBr) (cm^{-1}): 3527 (m), 3292 (br s), 3034 (w), 2944 (m), 1682 (br s), 1536 (m), 1497 (s), 1452 (s), 1401 (s), 1362 (m), 1267 (s), 1160 (s), 751 (m), 701 (m)

500 MHz ^{1}H NMR ($CDCl_3$): δ 8.96-8.72 (br m), 7.10-7.50 (br m), 6.55 (br m), 5.45 (br m), 5.10 (br m), 4.85 (br m), 4.60-4.40 (br m), 4.30-4.05 (br m), 4.0-3.80 (br m), 3.50-3.25 (br m), 3.15 (br m), 2.95-2.85 (br m), 2.65 (br m), 1.90 (br m), 1.70 (br m), 1.55 (br m), 1.4 (br m)

125 MHz ^{13}C NMR ($CDCl_3$): δ 174.2, 169.9, 169.1, 167.2, 156.3, 156.1, 136.8, 136.0, 135.7, 134.3, 130.0, 129.8, 129.1, 128.7, 128.3, 128.1, 82.0, 81.3, 78.1, 68.0, 67.6, 59.2, 56.3, 54.5, 52.0, 49.9, 49.4, 45.1, 45.0, 41.9, 36.7, 35.6, 28.3, 27.2, 25.5, 23.2

FAB HRMS: Calcd. for $C_{32}H_{43}O_9N_7Na$ $(M+Na)^+$: 692.3020; Found: 692.3040

Optical Rotation: $[\alpha]_D = -115.6^0$ (*c* 0.09, CH_2Cl_2)



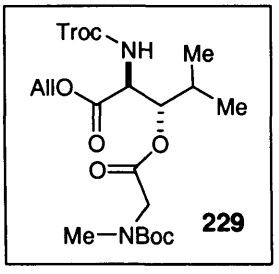
228

To sarcosine (5 g, 56 mmol) in 2M aq. NaOH (28 ml, 56 mmol) and THF (15 ml) at 0 ^{0}C was added dropwise over 10 min a solution of Boc_2O (12.25 g, 56 mmol) in THF (10 ml). The reactants were allowed to stir at 0 ^{0}C for 24 h after which Et_2O (50 ml) was added and the aqueous layer removed. The aqueous layer was acidified to pH 2 with conc. HCl and extracted with Et_2O (4 x 50 ml). The combined Et_2O layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford **228** as a clear oil that solidified to afford a white solid (Yield: 7 g, 66%).

500 MHz ^{1}H NMR ($CDCl_3$): δ 9.0 (br s, 1H), 3.97 (2 x s, 2H), 2.91 (s, 3H), 1.46 (2 x s, 9H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 174.7, 156.3, 155.6, 80.6, 50.6, 50.0, 35.4, 28.2

FAB HRMS: Calcd. for: $C_8H_{15}O_4NNa$ $(M+Na)^+$: 212.0899; Found: 212.0904



229

To **92** (4.39 g, 12 mmol), and *N*-Boc-sarcosine **228** (2.75 g, 14.5 mmol) in dry CH_2Cl_2 (70 ml) at RT and under N_2 was added DCC (2.64 g, 12.8 mmol) and DMAP (1.46 g, 12 mmol). The reactants were allowed to stir for 24 h after which CH_2Cl_2 (70 ml) was added and the solid that had formed during the course of the reaction was removed by filtration. The filtrate was concentrated *in vacuo* and the product purified by SiO_2 flash chromatography (1:0, 11:1, 3:1 hexanes/EtOAc) to afford **229** as a clear viscous oil (Yield: 5.6 g, 87%).

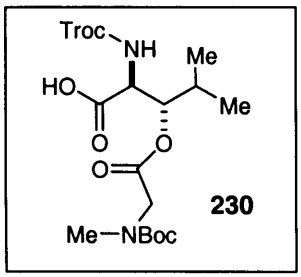
Infra-Red (neat) (cm^{-1}): 3323 (br w), 2974 (m), 2936 (w), 2880 (w), 1747 (br s), 1699 (br s), 1537 (br m), 1481 (m), 1454 (m), 1393 (s), 1367 (m), 1300 (w), 1248 (br s), 1177 (br s), 1151 (s)

500 MHz ^{1}H NMR ($CDCl_3$): δ 6.05 and 5.80 (2 x br d, J = 8.3,7.5 Hz, 1H), 5.89 (m, 1H), 5.35 (br d, J = 17.1 Hz, 1H), 5.26 (apparent t, J = 9 Hz, 1H), 4.93 (m, 1H), 4.70 (m, 2H, 1H), 4.63 (br d, J = 5.7 Hz, 2H), 4.0-3.87 (2 x d, J = 17.6 Hz, s, 2H), 2.89 (s, 3H), 2.08 (m, 1H), 1.41 (2 x s, 9H), 1.02 (m, 3H), 0.91 (m, 3H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 170.4, 170.2, 168.9, 168.8, 156.5, 155.6, 154.6, 154.4, 131.6, 131.5, 119.9, 119.7, 95.7, 95.6, 80.7, 80.1, 80.0, 75.1, 67.0, 66.9, 55.9, 51.1, 51.0, 36.2, 35.9, 29.7, 28.7, 19.5, 18.9

FAB HRMS: Calcd. for: $C_{20}H_{31}O_8N_2Cl_3Na$ $(M+Na)^+$: 555.1044; Found: 555.1030

Optical Rotation: $[\alpha]_D$ = -9.2 ^{0} (*c* 0.27, CH_2Cl_2)



230

To depsipeptide **229** (1.3 g, 2.4 mmol) in dry THF (15 ml) at RT under N_2 was added morpholine (1.77 ml, 20.4 mmol) and $(Ph_3P)_4Pd$ (0.2 g, 0.24 mmol). The reactants were allowed to stir for 20 min and diluted with Et_2O (70 ml), washed with 1 M HCl (3 x 40 ml) and brine (50 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (4:1 hexanes/EtOAc) to afford **230** as a white foam (Yield: 0.9 g, 75%).

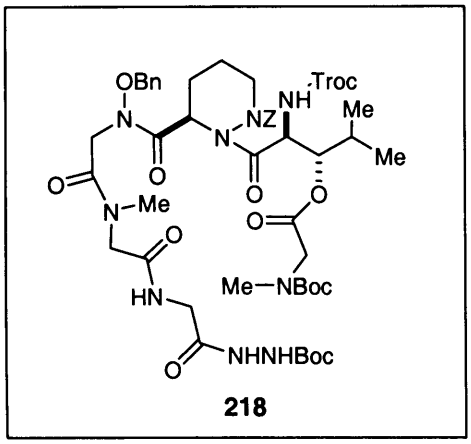
Infra-Red (KBr) (cm^{-1}): 3427 (br w), 2976 (m), 2937 (w), 1744 (br s), 1686 (br m), 1483 (w), 1458 (w), 1394 (m), 1369 (w), 1251 (br s), 1153 (s)

500 MHz ^{1}H NMR ($CDCl_3$): δ 9.85 (br s, 1H), 6.5 and 6.0 (d, *J* = 8.3, 8.0 Hz, 1H), 4.97 (m, 1H), 4.68 (m, 2H, 1H), 4.2-3.7 (2 x d, *J* = 17.6 Hz, s, 2H), 2.85 (s, 3H), 2.17 (m, 1H), 1.39 (2 x s, 9H), 1.5 (m, 3H), 0.89 (m, 3H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 171.3, 171.2, 169.5, 169.4, 156.4, 155.7, 154.2, 154.0, 95.2, 95.1, 81.1, 81.0, 80.0, 79.6, 74.9, 74.6, 55.1, 55.0, 50.8, 50.3, 50.2, 35.7, 35.5, 29.3, 29.1, 28.2, 18.9, 18.5

FAB HRMS: Calcd. for: $C_{17}H_{27}O_8N_2Cl_3Na$ $(M+Na)^+$: 515.0731; Found: 515.0720

Optical Rotation: $[\alpha]_D$ = +10.7 ^{0} (*c* 1.2, CH_2Cl_2)



218

To depsipeptide **230** (0.76 g, 1.49 mmol) in dry C_6H_6 (5 ml) at RT under N_2 was added $(COCl)_2$ (4.7 ml, 50.4 mmol) and allowed to stir at this temperature for 2.5 h. The reaction mixture was concentrated *in vacuo* and co-evaporated with dry C_6H_6 (2x8 ml) to remove the excess $(COCl)_2$. The resulting yellow foam **220** was dissolved in dry C_6H_6 and added to tetrapeptide **227** (1 g, 1.49 mmol) at RT and under N_2. With stirring and maintaining the N_2 atmosphere, AgCN (0.3 g, 2.24 mmol) was added in one portion. The reaction vessel was fitted with a reflux condenser, and immersed in an oil bath, pre-heated to 70 ^{0}C, for 10 min, with the apparatus covered in aluminium foil and the system under N_2. The reaction mixture was diluted with EtOAc (50 ml), filtered through Celite, and the Celite washed thoroughly with EtOAc. The filtrate was concentrated *in vacuo* and the product purified by SiO_2 flash chromatography (1:1 then 0:1 hexanes/EtOAc) to afford **218** as a white foam (Yield: 1.38 g, 81% 2 steps).

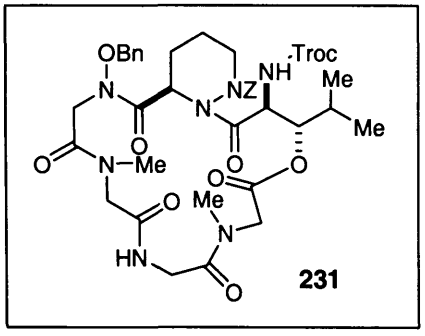
500 MHz ^{1}H NMR ($CDCl_3$): δ 8.55 (br m), 7.50-7.15 (br m), 6.95-6.75 (br m), 5.75 (br m), 5.29-4.90 (br m), 4.85-4.30 (br m), 4.20-3.55 (br m), 3.10 (br m), 2.95-2.80 (br m), 2.20 (br m), 1.95 (br m), 1.40-1.30 (br m), 0.95 (br m)

125 MHz ^{13}C NMR ($CDCl_3$): δ 172.2, 171.1, 169.0, 166.5, 156.2, 156.0, 155.7, 155.3, 154.2, 153.4, 135.9, 134.2, 132.0, 130.0, 129.1, 129.0, 128.3, 128.0, 127.1, 95.4, 95.1, 81.5, 80.1, 78.2, 74.6, 74.4, 69.0, 68.3, 52.0, 51.9, 50.9, 50.1, 49.2, 46.3, 46.0, 41.7, 41.2, 36.1, 35.4, 29.0, 28.3, 25.2, 20.5, 18.0, 17.5, 16.9

Infra-Red (KBr) (cm^{-1}): 3325 (br w), 2967 (w), 2933 (m), 1743 (br s), 1676 (br s), 1497 (m), 1452 (m), 1390 (s), 1362 (m), 1239 (br s), 1155 (br s)

FAB HRMS: Calcd. for: $C_{49}H_{68}O_{16}N_9Cl_3Na$ $(M+Na)^+$: 1166.3747; Found: 1166.3720

Optical Rotation: $[\alpha]_D$ = -15.3 ^{0} (*c* 0.75, CH_2Cl_2)



231

To hexadepsipeptide **218** (5.2 g, 4.54 mmol) in dry CH_2Cl_2 (70 ml) at RT under N_2 was added in one portion CF_3CO_2H (70 ml, 200 mmol). The reaction mixture was allowed to stir for 2 h, concentrated *in vacuo* and then co-evaporated with PhMe (2 x 30 ml) to remove the excess CF_3CO_2H. To the resulting residue in THF (55 ml) and H_2O (55 ml) was added NBS (1.6 g, 9 mmol) portionwise over 10 min. The reaction mixture was left to stir for 2 h, diluted with EtOAc (150 ml) and washed with brine (2 x 100 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield a white solid. To a suspension of HATU (4.07 g, 10.7 mmol) in dry CH_2Cl_2 (1.25l) at 0 ^{0}C and under N_2 was added dropwise over 8 h a solution of the above solid (1 g,

1.07 mmol) and *N*-ethylmorpholine (1.84 ml, 14.5 mmol) in dry CH_2Cl_2 (1.25l). After the addition the reaction mixture was stirred at 0 ^{0}C for 3 h and then RT for 48 h. The reaction mixture was washed with 1 M aq. HCl (2 x 750 ml), 5% aq. $NaHCO_3$ (750 ml) and brine (750 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (1:1, 0:1 hexanes/EtOAc) to afford **231** as a white solid (Yield: 0.66 g, 67% 3 steps)

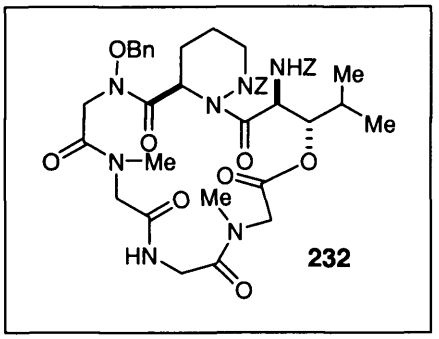
Infra-Red (KBr) (cm^{-1}): 3415 (br m), 2955 (m), 2877 (w), 1738 (s), 1671 (br s), 1530 (m), 1491 (m), 1452 (m), 1401 (m), 1345 (m), 1301 (m), 1239 (m), 1188(m), 1138 (w), 1116 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 7.45-7.10 (br m), 6.97 (br m), 6.60 (br m), 6.10-5.90 (br m), 5.6 (br m), 5.30-4.55 (br m), 4.45 (br m), 4.40-4.15 (br m), 4.15-3.60 (br m), 3.25 (q), 3.15-2.95 (br m), 2.95-2.70 (br m), 2.55 (br m), 2.45 (br m), 2.25 (br m), 2.0-1.30 (br m), 1.0-0.7 (br m)

125 MHz ^{13}C NMR ($CDCl_3$): δ 172.0, 171.2, 170.7, 169.8, 169.0, 168.3, 167.2, 166.7, 156.0, 155.5, 154.1, 153.5, 135.6, 132.7, 131.1, 130.1, 130.0, 129.3, 129.0, 128.7, 128.3, 96.0, 95.7, 95.0, 79.9, 76.0, 75.1, 74.3, 69.7, 69.2, 53.9, 53.5, 52.4, 52.0, 51.1, 50.3, 50.1, 49.8, 49.4, 49.3, 48.1, 47.9, 47.1, 43.0, 40.6, 40.2, 37.5, 36.7, 35.2, 29.1, 28.9, 28.8, 25.5, 25.1, 20.5, 19.7, 19.3, 18.1, 16.3, 16.1

FAB HRMS: Calc for $C_{39}H_{48}O_{12}N_7Cl_3Na$ $(M+Na)^+$: 934.2324; Found: 934.2300

Optical Rotation: $[\alpha]_D$ = -105.8 ^{0} (*c* 0.15, CH_2Cl_2)



232

To cyclodepsipeptide **231** (1.2 g, 1.32 mmol) in a solution of AcOH: H_2O (10:1, 30 ml) at RT was added Zn dust (5.6 g, 85.8 mmol). The reaction mixture was left to stir vigorously for 45 min, diluted with THF and filtered through Celite. The Celite was washed well with THF and the filtrate concentrated *in vacuo*. To the resulting residue was added dropwise and simultaneously a solution of Z-Cl (0.57 ml, 3.96 mmol) in CH_2Cl_2 (10 ml) and 10% aq. $NaHCO_3$ (10 ml) over 3 min. The reaction mixture was left to stir at RT for 1.5 h, diluted with EtOAc (30 ml) and washed with brine (15 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (EtOAc) to afford **232** as a white solid. (Yield: 880 mg, 77% 2 steps).

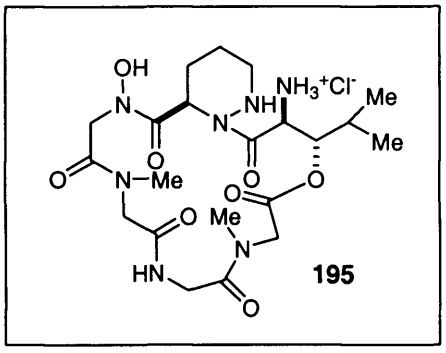
Infra-Red (KBr) (cm^{-1}): 3420 (br m), 3033 (w), 2965 (w), 2881 (w), 1731 (s), 1675 (br), 1528 (m), 1498 (m), 1455 (m), 1405 (m), 1347 (m), 1302 (m), 1237 (m), 1192 (m), 1136 (w) 1027 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 7.50-7.15 (br m), 7.14 (br m), 7.10 (br m), 6.95 (br m), 6.60 (br m), 5.89 (br m), 5.60 (br m), 5.30-4.55 (br m), 4.55-4.20 (br m), 4.10-3.70 (br m), 3.55 (br m), 3.35 (d), 3.23 (d), 3.05 (br s), 2.90 (br m), 2.78 (br m), 2.60 (br m), 2.45 (br m), 2.25 (br m), 2.05-1.70 (br m), 1.70-1.30 (br m), 1.05-0.8 (br m)

125 MHz ^{13}C NMR ($CDCl_3$): δ 172.3, 172.0, 170.8, 170.0, 169.5, 168.8, 168.1, 167.9, 166.5, 162.2, 161.0, 155.8, 155.2, 137.2, 136.3, 136.1, 135.7, 135.1, 134.5, 132.3, 131.0, 130.1, 129.9, 129.4, 129.1, 128.9, 128.7, 128.1, 127.2, 80.1, 78.2, 76.3, 69.5, 68.9, 67.1, 66.0, 54.0, 53.4, 52.5, 52.0, 51.4, 51.3, 50.1, 49.9, 49.7, 49.0, 47.8, 47.0, 43.1, 40.8, 40.5, 37.2, 36.7, 35.2, 33.1, 29.2, 29.0, 28.7, 25.3, 25.1, 20.4, 20.2, 19.4, 18.9, 18.5, 16.5, 16.1

FAB HRMS: Calcd. for: $C_{44}H_{53}O_{12}N_7Na$ $(M+Na)^+$: 894.3650; Found: 894.3632

Optical Rotation: $[\alpha]_D$ = -103.3 ^{0} (*c* 0.21, CH_2Cl_2)



195

To cyclodepsipeptide **232** (0.88 g, 1.01 mmol), in dry methanolic HCl (0.01 M, 101 ml, 1.01 mmol) was added 10% wet Pd/C (575 mg) and the system purged with H_2. The reaction mixture was left to stir vigorously at RT for 24 h, diluted with MeOH (70 ml) and filtered through Celite. The Celite pad was washed well with MeOH and the filtrate concentrated *in vacuo* to yield **195** as a white solid (Yield: 555 mg, 100%).

The 0.01 M solution of dry methanolic HCl was prepared by adding AcCl (0.25 ml) dropwise to dry MeOH (351 ml) and stirring at RT for 10 min before use.

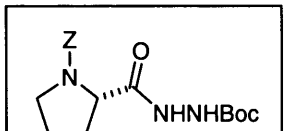
Infra-Red (KBr) (cm^{-1}): 3423 (br s), 3264 (s), 2967 (s), 2935 (s), 2872 (m), 1746 (s), 1656 (br s), 1545 (m), 1492 (s), 1417 (s), 1338 (m), 1301 (m), 1242 (s), 1200 (m), 1147 (w), 1120 (m), 1020 (m), 993 (m), 956 (w), 919 (w), 871 (w), 823 (w), 744 (w), 680 (w), 622 (w), 585 (w), 558 (w)

500 MHz ^{1}H NMR (CD_3OD): **δ** 5.38 (d, *J* = 15.3 Hz, 1H), 5.27 (br s, 1H), 5.13 (br d, *J* = 5.8 Hz, 1H), 5.08 (br d, *J* = 9.9 Hz, 1H), 5.01 (d, *J* = 17.7 Hz, 1H), 4.45 (d, *J* = 17.4 Hz, 1H), 4.0 (d, *J* = 17.0 Hz, 1H), 3.91 (d, *J* = 17.4 Hz, 1H), 3.87 (d, *J* = 17.0 Hz, 1H), 3.81 (d, *J* = 15.3 Hz, 1H), 3.75 (d, *J* = 17.7 Hz, 1H), 3.15 (s, 3H), 3.10 (m, 1H), 2.87 (s, 3H), 2.73 (dt, *J* = 12.6, 3.3 Hz, 1H), 2.18 (br d, *J* = 13.7 Hz, 1H), 1.98 (m, 1H), 1.84 (m, 1H), 1.7-1.45 (m, 2H), 1.16 (d, *J* = 6.7 Hz, 3H), 0.91 (d, *J* = 6.7 Hz, 3H)

125 MHz ^{13}C NMR (CD_3OD): δ 173.7, 172.4, 172.0, 169.9, 169.2, 169.1, 78.4, 53.4, 52.7, 52.3, 51.1, 50.5, 47.5, 42.7, 37.3, 34.9, 30.3, 24.2, 21.8, 19.3, 19.2

FAB HRMS: Calcd. for: $C_{21}H_{36}O_8N_7$ $(M+H)^+$: 514.2625; Found: 514.2600

Optical Rotation: $[\alpha]_D$ = -64.4 ^{0} (*c* 0.12, MeOH)



236

To *N*-Z-L-proline **235** (10 g, 40 mmol) in dry THF (115 ml) at 0 ^{0}C and under N_2, was added in sequence DCC (9.1 g, 44 mmol) and $BocNHNH_2$ (6.37 g, 48 mmol). The reaction mixture was left to stir at 0 ^{0}C for 1 h and then RT for 24 h, after which Et_2O (300 ml) was added and the solid residue filtered. The filtrate was washed with 1M HCl (2 x 100 ml), 5% aqueous solution of $NaHCO_3$ (2 x 100 ml), and brine (2 x 100 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (1:1, 1:3, hexanes/EtOAc) to afford **236** as a white foam (Yield: 12.3 g, 84%).

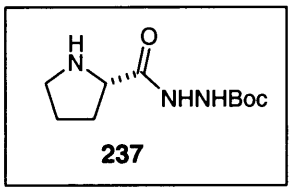
Infra-Red (KBr) (cm^{-1}): 3289 (s), 3029 (m), 2974 (s), 2878 (m), 1733 (s), 1713 (s), 1692 (s), 1672 (s), 1514 (m), 1446 (m), 1418 (m), 1357 (m), 1240 (m), 1158 (m), 1124 (m), 1089 (w), 1048 (w), 1021 (w), 987 (w), 911 (w), 870 (w), 767 (w), 726 (w), 692 (w), 644 (w), 610 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 8.6, 8.2 (br s, 1H), 7.30 (m, 5H), 6.75, 6.50 (br s, 1H), 5.10 (2d, AB system, *J* = 12.8 Hz, 2H), 4.40 (br s, 1H), 3.40 (m, 2H), 2.4-1.8 (m, 4H), 1.40 (s, 9H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 171.3, 156.0, 155.2, 136.2, 128.5, 128.4, 128.3, 128.0, 127.9, 127.7, 125.5, 81.6, 81.3, 67.4, 67.0, 59.6, 58.9, 47.4, 47.0, 31.0, 28.7, 28.3, 28.1, 24.9, 23.6

FAB HRMS: Calcd. for $C_{18}H_{26}O_5N_3$ $(M+H)^+$:364.1872; Found: 364.1860

Optical Rotation: $[\alpha]_D = -75.6^0$ (*c* 0.58, CH_2Cl_2)



237

To **236** (11.4 g, 30 mmol) in MeOH (60 ml) at RT was added 20% $Pd(OH)_2$ on carbon (0.32 g, 3 mmol). The system was purged with H_2 and stirred vigorously at RT for 12 h. The reaction mixture was diluted with MeOH (200 ml) and filtered through a pad of Celite. The filter pad was washed well with MeOH, and the filtrate concentrated *in vacuo* to yield **237** as a white solid (Yield: 7.2 g, 100%).

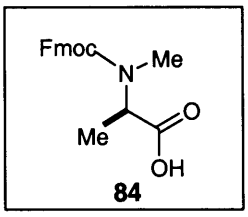
Infra-Red (KBr) (cm^{-1}): 3286 (s), 2975 (s), 2918 (m), 2864 (m), 1726 (s), 1681 (s), 1485 (m), 1452 (m), 1392 (m), 1367 (m), 1295 (w), 1245 (m), 1163 (s), 1099 (w), 1046 (w), 1016 (w), 919 (w), 867 (w), 732 (w), 646 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 3.80 (dd, *J* = 9.2 Hz, 5.3 Hz, 1H), 2.90 (m, 2H), 2.10 (m, 1H), 1.90 (m, 1H), 1.72 (m, 1H), 1.65 (m, 1H), 1.40 (s, 9H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 174.0, 155.1, 81.3, 59.7, 47.1, 30.5, 28.1, 26.0

FAB HRMS: Calcd. for $C_{10}H_{20}O_3N_3$ $(M+H)^+$: 230.1505; Found: 230.1492

Optical Rotation: $[\alpha]_D = -55.7^0$ (*c* 0.49, CH_2Cl_2)



84

To D-alanine (10 g, 110 mmol) in dioxane (110 ml) at 0 ^{0}C was added 10% aq. Na_2CO_3 (230 ml), followed by a solution of Fmoc-Cl (31.6 g, 122 mmol) in dioxane (110 ml) dropwise over 30 min. The mixture was allowed to stir at 0 ^{0}C for 30 min and then RT for 2 h. The reaction mixture was diluted with H_2O (400 ml) and washed with Et_2O (2 x 350 ml). The aqueous layer was acidified to pH2 with concentrated HCl and extracted with EtOAc (3 x 400 ml). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting foam was dissolved in dry toluene (400 ml), and TsOH (1.7 g, 9 mmol) and paraformaldehyde (22 g) were added. The reaction flask was fitted with a reflux condenser and Dean-Stark apparatus and the mixture was heated at reflux in an oil bath (115 ^{0}C) for 1 h. The reaction mixture was allowed to cool and diluted with EtOAc (400 ml) and washed with 10% aq. $NaHCO_3$ (300 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield a white solid. The white solid obtained was dissolved in chloroform (29 ml) and to the solution at RT was added CF_3CO_2H (29 ml) and Et_3SiH (7 ml, 44 mmol). The reactants were allowed to stir for 16 h at RT and under N_2. The reaction mixture was concentrated *in vacuo* and co-evaporated from PhMe (3 x 10 ml) until a white solid was obtained. The product was recrystallised from Et_2O/EtOAc to give **84** as a white powder (23.8 g, 65% 3 steps)

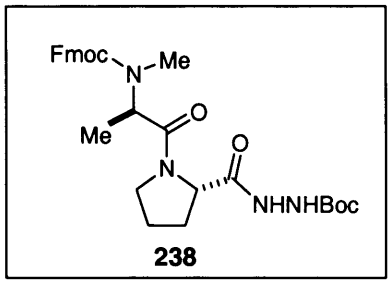
Infra-Red (KBr) (cm^{-1}): 3453 (m), 3070 (s), 2933 (s), 1740 (s), 1699 (s), 1651 (s), 1480 (m), 1446 (s), 1404 (m), 1315 (m), 1247 (w), 1199 (w), 1158 (m), 1096 (w), 1062 (w), 1000 (w), 904 (w), 802 (w), 760 (m), 733 (m), 644 (w)

500 MHz ^{1}H NMR ($CDCl_3$): (2:1 ratio of confomers) **δ** 11.1 (br s, 1H), 7.80 (br m, 2H), 7.60 (br m, 2H), 7.40 (br m, 2H), 7.30 (br m, 2H), 4.92 (q, *J* = 7 Hz, 0.66H) 4.63 (q, *J* = 7.2 Hz, 0.33H), 4.45 (m, 2H), 4.27 (t, *J* = 6.8 Hz, 0.66H) 4.21 (t, *J* = 6.5 Hz, 0.33H), 2.90 (s, 3H), 1.47 (d, *J* = 7 Hz, 2H) 1.37 (d, *J* = 7.2 Hz, 1H)

125 MHz ^{13}C NMR ($CDCl_3$): (major and (minor) confomers) δ 177.5, (177.4), 156.8, (156.0), 143.9, (143.8), 141.3, 127.7, 127.1, 125.0, (124.8), 120.0, 67.9, (67.8), 54.2, (53.9), 47.2, (30.7), 30.5, (15.0), 14.6

FAB HRMS: Calcd. for $C_{19}H_{20}O_4N$ $(M+H)^+$: 326.1392; Found: 326.1408

Optical Rotation: $[\alpha]_D$ = +21.4 ^{0} (*c* 0.49, CH_2Cl_2)



238

To the acid **84** (9.0 g, 28 mmol) in dry CH_2Cl_2 (85 ml) under N_2 was added $(COCl)_2$ (85 ml, 0.97 mol) in one portion at RT, where it was allowed to stir for 2 h. The reaction mixture was concentrated *in vacuo* and the residue coevaporated with C_6H_6 (2 x 8 ml) to remove the excess $(COCl)_2$. The resulting yellow foam **85** was kept under high vacuum for 30 min. To this yellow foam was added a solution of amine **237** (6.34 g, 28 mmol) in dry C_6H_6 (91 ml) at RT under N_2, and with stirring AgCN (5.57 g, 42 mmol) was added in one portion. The reaction vessel was fitted with a reflux condenser, covered with aluminium foil and heated to 80 ^{0}C for 45 min under N_2. The reaction mixture was cooled and diluted with EtOAc (50 ml), filtered through Celite, and the Celite washed thoroughly with EtOAc. The filtrate was concentrated *in vacuo* and the product purified by SiO_2 flash chromatography (2:1, 1:1, 1:2 hexanes/EtOAc) to afford **238** as a white foam (Yield: 13.8 g, 93% 2 steps).

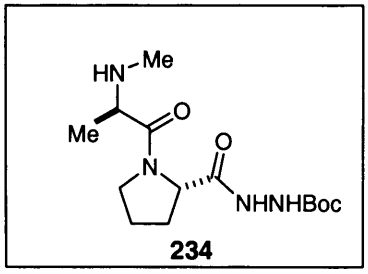
Infra-Red (KBr) (cm^{-1}): 3280 (s), 3061 (w), 2979 (m), 2924 (w), 2869 (w), 1736 (m), 1695 (s), 1633 (s), 1448 (s), 1400 (m), 1366 (m), 1318 (m), 1297 (m), 1243 (m), 1154 (s), 1078 (w), 1051 (w), 1017 (w), 907 (w), 873 (w),756 (m), 736 (s), 653 (w), 633 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 8.70 (br s), 8.45 (br s), 7.75 (br m, 2H), 7.55 (br m, 2H), 7.40 (br m, 2H), 7.30 (br m, 2H), 6.60-6.40 (br s, 1H), 4.75 (q, *J* = 6.8 Hz, 1H), 4.55 (m, 1H), 4.45 (d, *J* = 6.9 Hz, 2H), 4.25 (t, *J* = 7.4 Hz, 1H), 3.5 (m, 2H), 2.90 (s), 2.65 (s), 2.25 (m, 1H), 2.05 (m, 2H), 1.90 (m, 1H), 1.41 (s, 9H), 1.31(d, *J* = 6.8 Hz), 0.90 (d, *J* = 6.5 Hz)

125 MHz ^{13}C NMR ($CDCl_3$): δ 171.1, 171.0, 170.2, 156.5, 155.0, 143.8, 143.7, 141.5, 141.3, 127.7, 127.1, 127.1, 125.0, 125.0, 124.3, 119.9, 81.3, 81.1, 67.7, 66.4, 59.0, 58.4, 53.6, 52.6, 47.4, 47.3, 47.0, 46.3, 30.0, 29.0, 28.1, 27.3, 25.3, 25.0, 13.8, 13.6

FAB HRMS: Calcd. for $C_{29}H_{37}O_6N_4$ $(M+H)^+$: 537.2713; Found: 537.2701

Optical Rotation: $[\alpha]_D$ = +32.0 ^{0} (*c* 0.53, CH_2Cl_2)



234

To dipeptide **238** (13 g, 24 mmol) in MeCN (200 ml) was added Et_2NH (100 ml, 0.96 mol) and stirred at RT for 25 min under N_2. The reaction mixture was diluted with EtOAc (100 ml) and concentrated *in vacuo* and the product purified by SiO_2 flash chromatography (1:0, 5:1 EtOAc/MeOH) to afford **234** as a white solid (Yield: 7.14 g, 94%).

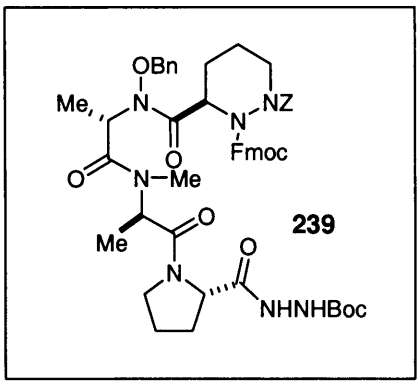
Infra-Red (KBr) (cm^{-1}): 3458 (m), 3280 (s), 2993 (s), 2938 (s), 2883 (m), 2787 (w), 1722 (m), 1695 (s), 1633 (s), 1455 (m), 1428 (m), 1393 (m), 1366 (m), 1311 (w), 1243 (m), 1167 (m). 1119 (w), 1078 (w), 1051 (w), 1017 (w), 921 (w), 866 (w), 804 (w), 729 (w), 646 (w), 633 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 4.5 (m, 1H), 3.7 (m, 1H), 3.4 (q, *J* = 8.9 Hz, 1H), 3.35 (q, *J* = 6.8 Hz, 1H), 2.3 (s, 3H), 2.3 (m, 1H), 2.13 (m, 1H), 1.95 (m, 2H), 1.40 (s, 9H), 1.41(d, *J* = 6 Hz), 1.17 (d, *J* = 6.8 Hz, 3H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 175.4, 171.1, 155.2, 80.9, 58.5, 56.6, 47.0, 34.3, 28.1, 27.9, 24.8, 18.1

FAB HRMS: Calcd. for $C_{14}H_{27}O_4N_4$ $(M+H)^+$: 315.2032; Found: 315.2016

Optical Rotation: $[\alpha]_D$ = -99.0 ^{0} (*c* 0.39, CH_2Cl_2)



239

To dipeptide **234** (1.48 g, 4.7 mmol) at RT under N_2 was added a solution of dipeptide **52** (3.0 g, 45 mmol) in dry CH_2Cl_2 (48 ml). The reaction mixture was cooled to -20 ^{0}C and dry Et_3N (1.25 ml, 9 mmol) followed by BOP-Cl (1.37 g, 5.4 mmol) were added. The reactants were allowed to stir at -20 ^{0}C for 20 min and then stirred at 0 ^{0}C for 1.5 h. The reaction mixture was diluted with EtOAc (250 ml), and washed with 0.5 M aq. HCl (2 x 150 ml), 5% aq. $NaHCO_3$ (2x150 ml) and brine (150 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (1:1, 5:1 hexanes/EtOAc) to afford **239** as a white foam (Yield: 3.86 g, 89%).

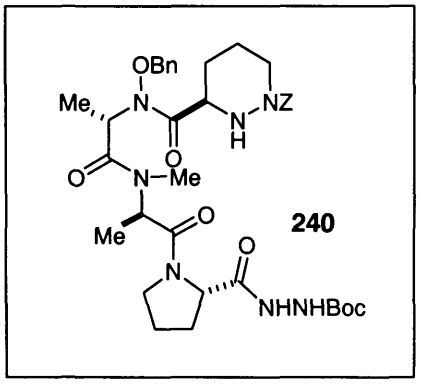
Infra-Red (KBr) (cm^{-1}): 3285 (s), 3062 (m), 2976 (s), 2927 (m), 2877 (m), 1703 (s), 1641 (s), 1449 (s), 1406 (s), 1363 (m), 1295 (m), 1245 (m), 1196 (m), 1159 (m), 1085 (w), 1041 (w), 1010 (w), 911 (w), 732 (m), 701 (w), 646 (w), 621 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 8.70-8.45 (br m), 7.10-7.80 (br m), 5.8 (br m), 5.55 (br m), 5.30-5.10 (br m), 5.05 (br m), 4.85 (br m), 4.6 (br m), 4.40-4.15 (br m), 3.95 (br m), 3.80 (br m), 3.65 (br m), 3.45-3.10 (br m), 3.10- 2.80(br m), 2.30 (br m), 2.15 (br m), 2.10-1.70 (br m), 1.55-1.20 (br m)

125 MHz ^{13}C NMR ($CDCl_3$): δ 172.3, 171.6, 171.4, 171.0, 170.6, 170.3, 156.7, 156.0, 156.6, 155.4, 154.9, 144.2, 143.6, 141.7, 136.9, 136.6, 134.9, 129.8, 129.1, 129.0, 128.9, 128.7, 128.5, 128.2, 127.5, 125.6, 125.5, 120.4, 120.3, 281.3, 81.1, 79.3, 79.0, 69.3, 69.1, 68.6, 68.3, 68.0, 59.6, 54.0, 53.7, 53.5, 50.5, 47.5, 47.4, 47.3, 45.6, 44.4, 32.1, 31.8, 31.4, 30.3, 28.8, 28.5, 25.0, 24.5, 24.2, 22.8, 19.5, 19.0, 15.4, 15.2, 14.6, 14.2, 14.1,

FAB HRMS: Calcd. for $C_{52}H_{61}O_{11}N_7Na$ $(M+Na)^+$: 982.4327; Found: 982.4302

Optical Rotation: $[\alpha]_D = -110^0$ (*c* 0.21, CH_2Cl_2)



To tetrapeptide **239** (3.56 g, 3.7 mmol) in MeCN (30 ml) was added Et_2NH (15 ml, 0.15 mol) and the reactants stirred at RT for 15 min under N_2. The reaction mixture was diluted with EtOAc and concentrated *in vacuo* and the product purified by SiO_2 flash chromatography (1:1, 0:1 hexanes/EtOAc) to afford **240** as a white solid (Yield: 2.73 g, 100%).

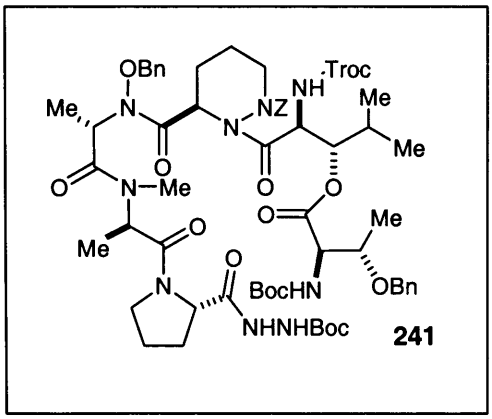
Infra-Red (KBr) (cm^{-1}): 3285 (m), 2976 (m), 2926 (m), 2877 (w), 1697 (s), 1641 (s), 1499 (m), 1449 (m), 1400 (m), 1369 (m), 1301 (w), 1257 (m), 1233 (m), 1158 (m), 1072 (w), 1004 (w), 917 (w), 868 (w), 787 (w), 725 (m), 701 (w), 639 (w), 608 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 8.65 (br m), 7.40-7.20 (br m), 6.45 (br m), 5.30-5.10 (br m), 5.20 (dd) 5.0 (br m), 4.60 (br m), 4.25 (br m), 4.15 (br m), 3.90 (br m), 3.80 (br m), 3.45 (br m), 3.15 (s), 2.95 (br m), 2.25 (br m), 2.15 (br m), 1.95 (br m), 1.80 (br m), 1.60 (br m), 1.50 (d), 1.40 (s), 1.35 (d)

125 MHz ^{13}C NMR ($CDCl_3$): δ 173.3, 171.5, 171.2, 156.3, 155.8, 155.6, 137.1, 137.0, 134.8, 129.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.5, 128.4, 128.4, 81.3, 80.0, 67.7, 59.8, 58.0, 57.1, 54.6, 52.2, 47.7, 47.6, 45.2, 31.5, 29.0, 28.6, 28.5, 27.5, 24.9, 23.7, 23.5, 14.9, 14.2, 14.1, 13.6

FAB HRMS: Calcd. for $C_{37}H_{51}O_9N_7Na$ $(M+Na)^+$: 760.3646; Found: 760.3615

Optical Rotation: $[\alpha]_D$ = -34 ^{0} (*c* 0.65, CH_2Cl_2)



241

To crude acid chloride **54** was added a solution of tetrapeptide **240** (2.56 g, 3.5 mmol) in dry C_6H_6 (30 ml) at RT and under N_2. With stirring, and whilst maintaining a N_2 atmosphere, AgCN (0.67 g, 5 mmol) was added in one portion. The reaction vessel was fitted with a reflux condenser, and immersed for 8 min in an oil bath pre-heated to 80 ^{0}C, the apparatus being covered in aluminium foil. The reaction mixture was cooled and diluted with EtOAc (50 ml), filtered through Celite, and the Celite pad washed thoroughly with EtOAc. The filtrate was concentrated *in vacuo* and the product purified by SiO_2 flash chromatography (2:1 hexanes/EtOAc) to afford **241** as a white foam (Yield: 3.23 g, 73% 2 steps).

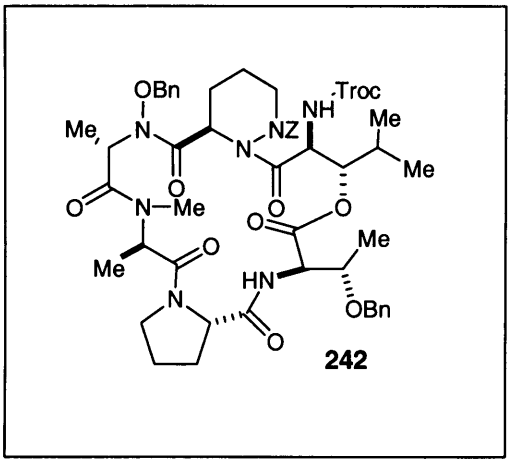
Infra-Red (KBr) (cm^{-1}): 3447 (m), 3299 (m), 2977 (m), 2940 (m), 2878 (w), 1728 (s), 1641 (s), 1499 (m), 1455 (m), 1394 (m), 1369 (m), 1350 (m), 1313 (m), 1239 (m), 1158 (m), 1078 (w), 1041 (w), 1041 (w), 991 (w), 911 (m), 824 (w), 732 (s), 701 (m), 651 (w), 571 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 8.80-8.40 (br m), 7.50-7.10 (br m), 6.05-6.60 (br m), 5.30-5.00 (br m), 4.95-4.75 (br m), 4.65-4.50 (br m), 4.35 (br m), 4.15 (br m), 3.85-3.40 (br m), 3.35 (br m), 3.1 (br m), 3.0 (br m), 2.95 (br m), 2.85 (br m), 2.70 (br m), 2.60-2.35 (br m), 2.25 (br m), 2.10-1.55 (br m), 1.50-1.30 (br m), 1.2 (d), 1.0-0.80 (br m)

125 MHz ^{13}C NMR ($CDCl_3$): δ 172.4, 172.0, 171.5, 170.1, 170.3, 170.0, 156.6, 156.3, 156.0, 155.8, 153.9, 138.2, 136.2, 134.5, 130.3, 129.8, 129.3, 129.2, 129.1, 128.7, 128.6, 128.0, 127.9, 127.8, 127.7, 127.1, 126.7, 95.7, 81.6, 81.5, 80.3, 79.3, 78.9, 75.1, 74.6, 71.1, 70.1, 69.2, 68.5, 59.5, 59.2, 58.6, 53.6, 51.5, 47.7, 47.2, 46.0, 29.1, 28.6, 28.5, 25.2, 25.0, 24.8, 20.2, 17.7, 16.8, 16.6, 16.4, 15.5, 14.5, 14.2

FAB HRMS: Calcd. for $C_{62}H_{84}O_{17}N_9Cl_3Na$ $(M+Na)^+$: 1354.4943; Found: 1354.4990

Optical Rotation: $[\alpha]_D$ = -85.6 ^{0} (*c* 0.27, CH_2Cl_2)



242

To hexadepsipeptide **241** (3.03 g, 2.3 mmol) in dry CH_2Cl_2 (35 ml) at 0 ^{0}C under N_2 was added in one portion CF_3CO_2H (35 ml, 0.46 mol). The reactants were allowed to stir for 2 h at 0 ^{0}C. The reaction mixture was concentrated *in vacuo* and then co-evaporated with PhMe (2 x 30

ml) to remove the excess CF_3CO_2H. To the resulting residue in THF (25 ml) and H_2O (25 ml) at RT was added NBS (0.81 g, 4.5 mmol) portionwise over 7 min. The reaction mixture was left to stir for 1.5 h at RT, diluted with EtOAc (100 ml) and washed with brine (2 x 100 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield a white foam. To a suspension of HATU (3.4 g, 8.9 mmol) in dry CH_2Cl_2 (1.04 L) at 0 ^{0}C and under N_2 was added dropwise over 6 h a solution of the above solid (1 g, 0.89 mmol) and *N*-ethylmorpholine (1.53 ml, 12 mmol) in dry CH_2Cl_2 (1.04 L). After the addition the reaction mixture was allowed to warm to RT and stirred for 60 h. The reaction mixture was concentrated *in vacuo* and the residue taken up in EtOAc and washed with 1 M aq. HCl (2 x 25 ml), 5% aq. $NaHCO_3$ (2 x 25 ml) and brine (30 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (1:1, 1:2 hexanes/EtOAc) to afford **242** as a white solid (Yield: 0.47 g, 48% 3 steps).

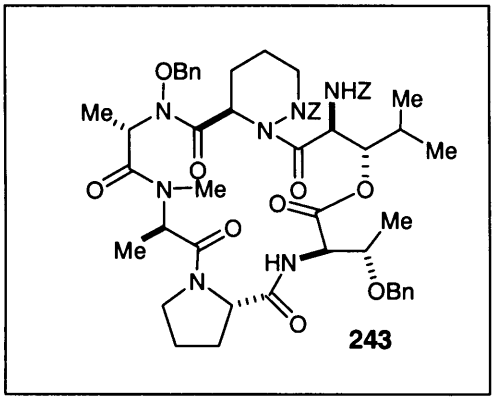
Infra-Red (KBr) (cm^{-1}): 3435 (m), 3287 (m), 3076 (w), 3027 (w), 2977 (m), 2928 (m), 2878 (m), 1740 (s), 1678 (s), 1641 (s), 1511 (m), 1455 (m), 1394 (m), 1344 (w), 1313 (w), 1257 (m), 1233 (m), 1202 (m), 1183 (m), 1152 (m), 1128 (m), 1078 (m), 1041 (w), 1004 (w), 911 (m), 825 (w), 732 (s), 694 (m), 651 (w), 620 (w), 564 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 7.50-7.15 (br m), 6.85-6.60 (br m), 5.65 (m), 5.45 (br m), 5.30-5.10 (br m), 5.05 (br m), 4.90 (br m), 4.80 (d), 4.70-4.40 (br m), 4.25-4.05 (br m), 3.95 (br m), 3.75 (br m), 3.60 (br m), 3.35 (br m), 3.20 (br m), 2.85 (br m), 2.80 (s), 2.10-1.80 (br m), 1.75 (br m), 1.60 (br m), 1.50 (d, J = 7.2 Hz), 1.45-1.1.35 (br m), 1.35 (d, J = 6.8 Hz), 1.30-1.15 (br m), 1.15 (d, J = 6.9 Hz), 1.0-0.65 (br m)

125 MHz ^{13}C NMR ($CDCl_3$): δ 171.7, 171.1, 170.5, 170.4, 170.0, 169.8, 158.7, 154.4, 137.8, 135.8, 135.5, 134.3, 130.0, 129.6, 129.4, 129.1, 128.9, 128.8, 128.7, 128.5, 128.3, 128.0, 128.0, 127.8, 95.6, 95.5, 79.1, 78.8, 75.3, 75.1, 74.9, 71.2, 69.5, 69.3, 62.1, 57.7, 56.9, 53.6, 52.5, 51.2, 48.0, 47.3, 45.7, 39.0, 31.0, 30.1, 29.2, 25.3, 20.0, 19.6, 19.2, 18.6, 17.6, 16.4, 16.0, 15.6, 15.1, 14.5, 14.3

FAB HRMS: Calcd. for $C_{52}H_{64}O_{13}N_7Cl_3Na$ $(M+Na)^+$: 1122.3525; Found: 1122.3550

Optical Rotation: $[\alpha]_D = -50.6^0$ (*c* 0.2, CH_2Cl_2)



243

To cyclodepsipeptide **242** (1.4 g, 1.3 mmol) in a solution of AcOH: H_2O (10:1, 30 ml) at RT was added Zn dust (7 g, 0.11 mol). The reaction mixture was left to stir vigorously for 20 min, diluted with THF and filtered through Celite. The Celite pad was washed well with THF and the filtrate concentrated *in vacuo*. To the resulting residue was added a solution of Z-Cl (0.6 ml, 14 mmol) in CH_2Cl_2 (14 ml) and 10% aq. $NaHCO_3$ (14 ml) dropwise and simultaneously over 6 min. The reactants were allowed to stir at RT for 1 h. The reaction mixture was diluted with EtOAc (50 ml) and washed with brine (20 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (3:1, 1:2 hexanes/EtOAc) to afford **243** as a white solid. (Yield: 1.07 g, 80% 2 steps).

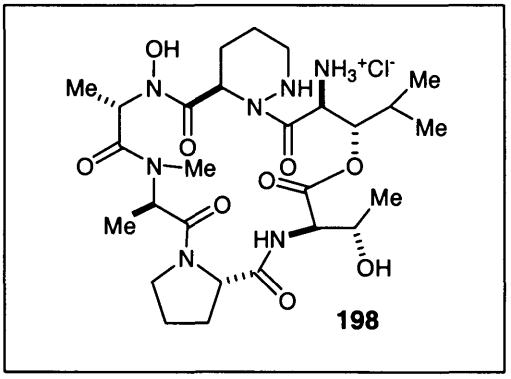
Infra-Red (KBr) (cm^{-1}): 3478 (m), 3404 (m), 3292 (m), 3032 (w), 2971 (m), 2933 (m), 2872 (w), 1731 (s), 1675 (s), 1644 (s), 1508 (m), 1452 (m), 1396 (m), 1328 (w), 1260 (m), 1229 (m), 1192 (w), 1137 (w), 1075 (w), 1038 (w), 1000 (w), 914 (w), 821 (m), 734 (m), 697 (m), 685 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 7.50-7.05 (br m), 6.80-6.50 (br m), 5.50-5.35 (br m), 5.25 (br m), 5.20-4.80 (br m), 4.65-4.30 (br m), 4.15 (br m), 3.95 (br m), 3.75 (br m), 3.35 (br m), 3.20 (br m), 2.80 (br m), 2.80 (s), 2.10-1.70 (br m), 1.55 (br m), 1.45 (d, *J* = 7.0 Hz), 1.25 (d, *J* = 7.0 Hz), 1.15 (d, *J* = 6.9 Hz), 1.0-0.65 (br m)

125 MHz ^{13}C NMR ($CDCl_3$): δ 171.6, 171.1, 170.6, 170.4, 170.3, 170.1, 169.8, 158.8, 155.8, 137.8, 136.6, 135.9, 134.3, 130.0, 129.5, 129.3, 129.1, 128.9, 128.9, 128.8, 128.7, 128.7, 128.6, 128.5, 128.1, 127.9, 127.5, 79.5, 78.8, 74.8, 70.8, 69.4, 69.0, 67.8, 67.6, 62.5, 56.9, 56.1, 53.5, 52.6, 51.1, 50.8, 48.0, 45.1, 39.0, 31.1, 30.1, 29.5, 29.2, 25.7, 25.3, 20.0, 19.5, 19.2, 18.8, 17.7, 16.4, 15.9, 15.0, 14.3, 14.1

FAB HRMS: Calcd. for $C_{57}H_{70}O_{13}N_7$ $(M+H)^+$: 1060.5032; Found: 1060.5057

Optical Rotation: $[\alpha]_D$ = -87.5 ^{0} (*c* 0.1, CH_2Cl_2)



198

To cyclodepsipeptide **243** (1.0 g, 0.94 mmol), in dry methanolic HCl (0.01 M, 94 ml, 0.94 mmol) was added 10% wet Pd/C (700 mg) and the system purged with H_2. The reaction mixture was left to stir vigorously at RT for 24 h, diluted with MeOH (70 ml) and filtered through Celite. The Celite pad was washed well with MeOH and the filtrate concentrated *in vacuo* to yield **198** as a white solid (Yield: 611m g, 100%).

Infra-Red (KBr) (cm^{-1}): 3417(br, s), 2969 (m), 2868 (m), 1746 (s), 1651(br, s), 1528 (m), 1444 (m), 1405 (m), 1371 (w), 1298 (m), 1254 (s), 1192 (w), 1170 (w), 1136 (w), 1114 (w), 1080 (m), 1002 (m), 918 (w), 845 (s), 789 (w), 605 (m), 554 (w)

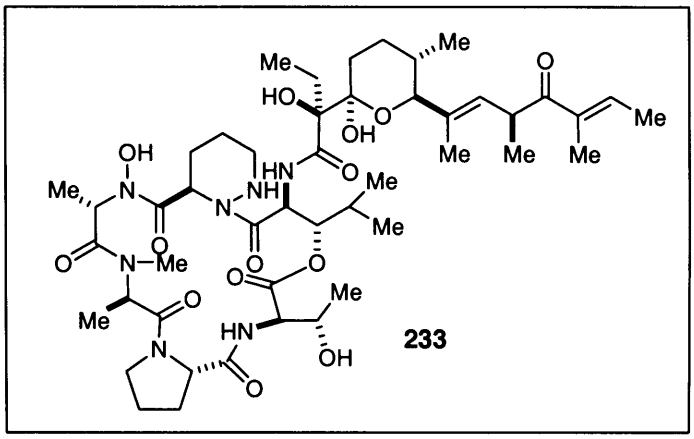
500 MHz ^{1}H NMR (CD_3OD): δ 5.55 (br m, 1H), 5.45 (br m, 1H), 5.35 (br m, 1H), 5.25 (br m, 1H), 5.05 (br m, 2H), 4.75 (s, 1H), 4.35 (br m, 2H), 3.95 (br m, 1H), 3.45 (br m, 1H), 3.15 (br m, 1H), 3.05 (br m, 1H), 3.0 (s, 3H), 2.85 (s, 1H), 2.30 (br m, 1H), 2.20 (br m, 1H), 2.0 (br m, 2H), 1.85 (br

m, 2H), 2.75 (br m, 1H), 2.65 (br m, 1H), 1.35 (d, J = 6.9 Hz, 3H), 1.30 (d, J = 7.1 Hz, 3H), 1.20 (d, J = 6.9 Hz, 3H), 1.05 (d, J = 6.8 Hz, 3H), 0.9 (d, J = 6.8 Hz, 3H)

125 MHz ^{13}C NMR (CD_3OD): δ 174.9, 173.1, 172.0, 171.7, 170.8, 168.7, 79.3, 70.7, 64.7, 58.4, 54.4, 54.3, 52.2, 50.5, 46.4, 31.8, 31.2, 30.9, 26.4, 23.2, 19.8, 19.5, 19.2, 18.9, 14.9, 14.8, 14.0

FAB HRMS: Calcd. for $C_{27}H_{46}O_9N_7$ $(M+H)^+$: 612.3357; Found: 612.3338

Optical Rotation: $[\alpha]_D$ = -31.7 ^{0} (*c* 0.2, MeOH)



233

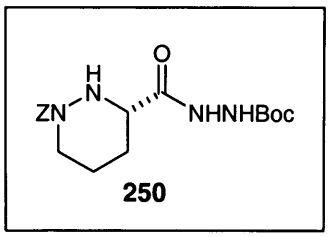
To cyclodepsipeptide salt **198** (0.11 g, 0.17 mmol) in dry CH_2Cl_2 (2 ml) under N_2 was added activated ester **47** (0.1 g, 0.2 mmol). The reaction was cooled to -78 ^{0}C and dry Et_3N (0.4 ml) was added dropwise over 2 min. The reaction was stirred at -78 ^{0}C for 10 min then at RT for 45 min. The reaction mixture was left in the freezer (-33 ^{0}C) overnight. The reaction was then stirred further at RT for 90 min before being diluted with EtOAc (50 ml). It was then washed successively with 1 M aq. HCl (25 ml), brine (25 ml), sat. aq. $NaHCO_3$ (25 ml) and more brine (25 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The product was purified by preparative TLC using 30:1 CH_2Cl_2: MeOH as the eluant and subsequently by SiO_2 flash chromatography (2:1 then 0:1, hexanes/EtOAc), to afford **233** as a white solid. (Yield: 28 mg, 17.3%)

Infra-Red (KBr) (cm^{-1}): 3407 (br s), 2967 (m), 2920 (m), 2860 (w), 1729 (m), 1663 (s), 1651 (s), 1634 (s), 1515 (m), 1449 (m), 1414 (m), 1378 (m), 1307 (m), 1265 (m), 1212 (w), 1182 (w), 1152 (w), 1069 (w), 1003 (w), 908 (w), 730 (m)

500 MHz ^{1}H NMR ($CDCl_3$): δ 9.25 (br s, 1H), 8.20 (d, *J* = 10.9 Hz, 1H), 6.70 (dq, *J* = 7.0 Hz, 1.3 Hz, 1H), 6.29 (br s, 1H), 5.93 (d, *J* = 9.9 Hz, 1H), 5.70 (q, *J* = 6.8 Hz, 1H), 5.60 (dd, *J* = 9.1 Hz, 1.3 Hz, 1H), 5.34 (dd, *J* = 10.8 Hz, 2.0 Hz, 1H), 5.20 (q, *J* = 7.2 Hz, 1H), 5.06 (dd, *J* = 6.9 Hz, 2.1 Hz, 1H), 4.89 (t, *J* = 10.8 Hz, 1H), 4.66 (m, 2H), 4.57 (dd, *J* = 8.5 Hz, 3.6 Hz, 1H), 4.52 (d, *J* = 12.4 Hz, 1H), 4.28 (br s, 1H), 4.14 (m, 1H), 4.05 (dd, *J* = 9.1 Hz, 6.9 Hz, 1H), 3.94 (d, *J* = 10.5 Hz, 1H), 3.59 (m, 1H), 3.12 (m, 1H), 3.11 (s, 3H), 2.95(m, 1H), 2.90 (s, 1H), 2.30-1.90 (m), 1.83(dd, *J* = 7.1 Hz, 1.0 Hz, 3H), 1.74 (br s, 3H), 1.68-1.60 (m), 1.55 (d, *J* = 1.2 Hz, 3H), 1.47 (d, *J* = 7.1 Hz, 3H), 1.45 (m), 1.32 (d, *J* = 6.9 Hz, 3H), 1.09 (d, *J* = 7.0 Hz, 3H), 1.04 (d, *J* = 6.5 Hz, 3H), 0.82 (t, *J* = 7.2 Hz, 3H), 0.70 (d, *J* = 6.8 Hz, 3H), 0.68 (d, *J* = 6.6 Hz, 3H), 0.63 (d, *J* = 7.2 Hz, 3H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 202.9, 175.3, 175.0, 174.4, 171.2, 170.5, 170.0, 168.8, 137.5, 136.7, 132.8, 129.4, 99.6, 82.2, 80.1, 78.1, 65.4, 60.7, 55.7, 54.5, 51.7, 50.7, 48.3, 47.9, 45.8, 38.2, 32.6, 29.7, 29.6, 28.9, 28.3, 27.3, 25.9, 24.4(x2), 21.1, 19.5, 19.4, 18.3, 17.6, 14.8, 14.3, 13.8, 13.1, 12.0, 11.4, 8.2

FAB HRMS: Calcd. for $C_{47}H_{75}O_{14}N_7Na$ $(M+Na)^+$: 985.5348; Found: 985.5378



250

To **249** (20 g, 75.7 mmol) in dry THF (250 ml) at 0 ^{0}C and under N_2, was added in sequence DCC (17.2 g, 83.9 mmol), HOBt (11 g, 83.9 mmol) and $BocNHNH_2$ (11.2 g, 83.9 mmol). The reaction mixture was left to stir at 0 ^{0}C for 1 h and then RT for 20 h, after which Et_2O (300 ml) was added and the solid residue filtered. The filtrate was washed with 1 M aq. HCl (2 x 100 ml), 5% aq. $NaHCO_3$ (2 x 80 ml), and brine (100 ml). The organic layer was dried over $MgSO_4$, filtered

and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (1:1 then 0:1, hexanes/EtOAc) to afford **250** as a white foam (Yield: 23 g, 80%).

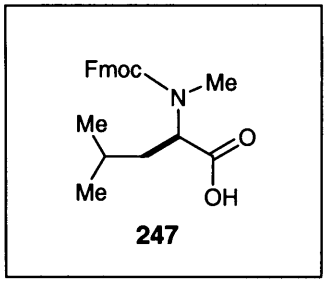
Infra-Red (KBr) (cm^{-1}): 3382 (s), 3302 (br, s), 3068 (w), 2977 (m), 1701 (br, s), 1451 (s), 1420 (s), 1392 (s), 1365 (s), 1301 (s), 1249 (s), 1197 (s), 1161 (s), 1127 (m), 1088 (m), 1049 (m), 975 (w), 911 (m)

500 MHz ^{1}H NMR ($CDCl_3$): δ 9.50 (br m, 2H), 7.35 (br m, 5H), 6.45 (br m, 1H), 5.10 (dd, 1H), 4.15 (br m, 1H), 3.95 (br m, 1H), 3.65 (br m, 1H), 3.10 (br m, 1H), 2.25 (br m, 1H), 1.70-1.55 (br m, 3H), 1.40 (s, 9H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 170.6, 155.8, 155.0, 136.1, 128.5, 128.3, 81.1, 67.9, 57.0, 44.8, 28.1, 25.0, 21.4

FAB HRMS: Calcd. for $C_{33}H_{36}O_7N_4Na$ $(M+Na)^+$: 623.2482; Found: 623.2446

Optical Rotation: $[\alpha]_D$ = -26.1 ^{0} (*c* 0.624, CH_2Cl_2)



247

To D-leucine **246** (10 g, 76 mmol) in dioxane (75 ml) at 0 ^{0}C was added 10% aq. Na_2CO_3 (162 ml), followed by a solution of Fmoc-Cl (21.4 g, 83 mmol) in dioxane (115 ml) dropwise over 35 min. The mixture was allowed to stir at 0 ^{0}C for 30 min and then RT for 2 h. The reaction mixture was diluted with H_2O (200 ml) and washed with Et_2O (2 x 250 ml). The aqueous layer was acidified to pH 2 with conc. HCl and extracted with EtOAc (3 x 300 ml). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting foam was dissolved in dry toluene (330 ml), and *p*-TsOH (1.16 g, 6.1 mmol) and paraformaldehyde (14.5 g) were added.

The reaction flask was fitted with a reflux condenser and Dean-Stark apparatus and the mixture was heated at reflux in an oil bath (125 ^{0}C) for 1 h. The reaction mixture was allowed to cool and diluted with EtOAc (300 ml) and washed with 10% aq. $NaHCO_3$ (200 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield a white solid. The white solid obtained was dissolved in chloroform (120 ml) and to the solution was added CF_3CO_2H (120 ml) and Et_3SiH (29 ml, 0.18 mol). This was left to stir for 24 h at RT and under N_2. The reaction mixture was concentrated *in vacuo* and further co-evaporated from PhMe (3 x 40 ml) until a white solid was obtained. The product was recrystallised from hexanes/EtOAc to form **247** as a white powder (18.1 g, 65% 3 steps)

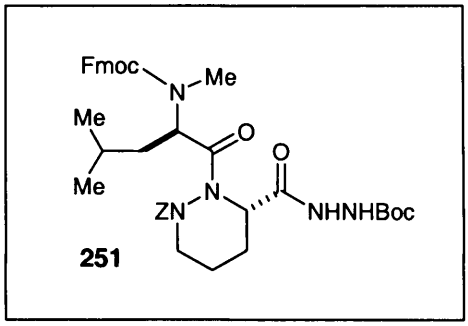
Infra-Red (KBr) (cm^{-1}): 2458 (br m), 2958 (s), 1709 (s), 1450 (m), 1403 (m), 1366 (w), 1319 (m), 1226 (w), 1162 (m), 1110 (w), 1071 (w), 1034 (w), 973 (w), 909 (m), 810 (w), 760 (m), 739 (s), 648 (w)

500 MHz ^{1}H NMR ($CDCl_3$): (2:1 ratio of confomers) **δ** 11.0 (br s, 1H), 7.75 (m, 2H), 7.55 (m, 2H), 7.4-7.2 (m, 4H), 4.90 (dd, *J* = 9.2 Hz, 7.1 Hz, 0.66H) 4.58 (q, *J* = 5.6 Hz, 0.33H), 4.45 (m, 2H), 4.26 (t, *J* = 7.1 Hz, 0.66H), 4.20 (t, *J* = 6.0 Hz, 0.33H), 2.90 (s, 2H), 2.85 (s, 1H), 1.73 (m, 1H), 1.55 (m, 2H), 0.95 (d, *J* = 6.7 Hz, 2H), 0.92 (d, *J* = 6.5 Hz, 2H), 0.86 (d, *J* = 6.7 Hz, 1H), 0.72 (d, *J* = 6.5 Hz, 1H)

125 MHz ^{13}C NMR ($CDCl_3$): (Mixture of major and (minor) confomers) **δ** 177.8, (177.6), 157.1, (156.5), (143.9), 143.8, 141.3, 127.6, 127.0, 125.0, (124.7), 119.9, 67.8, (67.6), 56.6, (56.4), 47.3, (47.2), (37.5), 37.2, (30.5), 30.3, 24.8, (24.7), 23.2, (23.0), 21.2, (21.0)

FAB HRMS: Calcd. for $C_{22}H_{26}NO_4$ $(M+H)^+$: 368.1862; Found: 368.1853

Optical Rotation: $[\alpha]_D$ = +22.5 ^{0} (*c* 1.1, CH_2Cl_2)



251

To the acid **247** (22.6 g, 61 mmol) in dry CH_2Cl_2 (112 ml) under N_2 was added $(COCl)_2$ (112 ml, 1.28 mol) in one portion at RT, where it was left to stir for 2 h. The reaction mixture was concentrated *in vacuo* and the residue was coevaporated with C_6H_6 (2 x 10 ml) to remove the excess $(COCl)_2$. The resulting yellow foam **248** was kept under high vacuum for 15 min. To this yellow foam was added a solution of amine **250** (23.5 g, 61 mmol) in dry C_6H_6 (200 ml) at RT under N_2, and whilst stirring and maintaining the N_2 atmosphere AgCN (12 g, 90 mol) was added in one portion. The reaction vessel was fitted with a reflux condenser, covered in aluminium foil and heated at 80 ^{0}C for 45 min under N_2. The reaction mixture was cooled and diluted with EtOAc (100 ml), filtered through Celite, and the Celite pad washed thoroughly with EtOAc. The filtrate was concentrated *in vacuo* and the product was purified by SiO_2 flash chromatography (3:1, 2:1 hexanes/EtOAc) to afford **251** as a white foam (Yield: 33 g, 71% 2 steps).

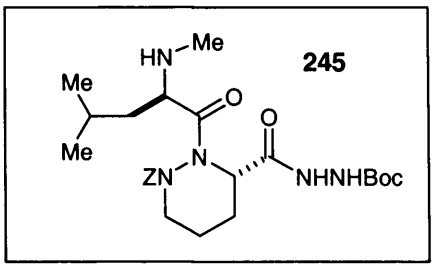
Infra-Red (KBr) (cm^{-1}): 3291 (m), 2957 (m), 1697 (s), 1451 (m), 1405 (m), 1365 (m), 1316 (m), 1247 (m), 1196 (w), 1160 (m), 1121 (w), 1044 (w), 912 (w), 759 (w), 737 (s), 700 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 9.40 (br m), 7.75 (br m), 7.55 (br m), 7.40-7.20 (br m), 6.20 (br m), 5.40-4.60 (br m), 4.45 (br m), 4.25 (br m), 3.15 (br m), 2.95 (s), 2.90-2.75 (br m), 2.40-1.50 (br m), 1.45 (2xs), 0.95 (dd), 0.80-0.70 (br m)

125 MHz ^{13}C NMR ($CDCl_3$): δ 174.1, 169.8, 168.1, 157.1, 156.3, 155.2, 154.8, 144.0, 143.8, 141.3, 135.2, 134.9, 129.9, 129.1, 128.9, 128.7, 128.5, 128.4, 128.3, 127.7, 127.5, 127.0, 126.9, 125.2, 125.1, 124.6, 119.9, 81.1, 69.7, 69.3, 67.5, 66.9, 66.7, 54.7, 52.5, 52.1, 47.3, 46.9, 46.6, 46.2, 38.3, 38.0, 29.7, 29.3, 28.1, 24.9, 24.6, 24.0, 23.3, 23.2, 23.0, 22.4, 22.0, 21.8, 20.1

FAB HRMS: Calcd. for $C_{40}H_{49}O_8N_5Na$ $(M+Na)^+$: 750.3479; Found: 750.3453

Optical Rotation: $[\alpha]_D$ = +16.8 ^{0} (*c* 0.28, CH_2Cl_2)



245

To dipeptide **251** (33 g, 45 mmol) in MeCN (400 ml) was added Et_2NH (200 ml, 1.81 mol) and the reactants stirred for 15 min at RT and under N_2. The reaction mixture was diluted with EtOAc and concentrated *in vacuo* and the product purified by SiO_2 flash chromatography (3:1, 0:1 hexanes/EtOAc) to afford **245** as a white solid (Yield: 20.5 g, 89%).

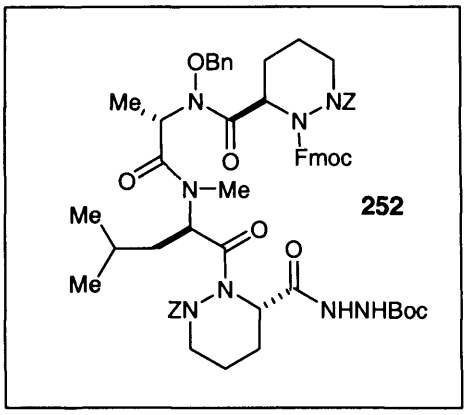
Infra-Red (KBr) (cm^{-1}): 3303 (m), 2957 (s), 2870 (m), 2804 (w), 1699 (br s), 1455 (s), 1404 (s), 1366 (s), 1246 (s), 1195 (s), 1164 (s), 1118 (m), 1088 (w), 1042 (m), 973 (w), 915 (m), 872 (w), 807 (w), 733 (s), 700 (m), 647 (w), 532 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 9.25 (br m), 7.35 (br m), 6.45 (br m), 5.25 (br m), 5.15 (br m), 4.25 (br m), 3.35-3.20 (br m), 3.10 (br m), 2.40 (br m), 2.25 (s), 2.05 (br m), 1.90-1.70 (br m), 1.55 (br m), 1.45 (s), 1.30 (br m), 1.05 (br m), 0.85 (br m), 0.75 (br m)

125 MHz ^{13}C NMR ($CDCl_3$): δ 178.5, 170.0, 157.0, 154.9, 135.2, 129.4, 128.6, 128.5, 81.1, 69.4, 58.1, 53.3, 46.7, 43.0, 35.0, 28.1, 24.9, 24.0, 23.6, 21.3, 20.4

FAB HRMS: Calcd. for $C_{25}H_{40}O_6N_5$ $(M+H)^+$: 506.2979; Found: 506.2964

Optical Rotation: $[\alpha]_D$ = -33.3 ^{0} (*c* 0.52, CH_2Cl_2)



252

To acid **52** (20 g, 30 mmol) in dry CH_2Cl_2 (200 ml) at -20 ^{0}C and under N_2, was added distilled collidine (4.5 ml, 33 mmol) and BOP-Cl (9.2 g, 36 mmol) and left to stir for 20 min at -20 ^{0}C. Amine **245** (15.3 g, 30 mmol) was added to the reaction mixture followed by distilled collidine (4.3 ml, 33 mmol) and left to stir at -20 ^{0}C for a further 20 min. The reaction mixture was warmed to RT and stirred for 16 h. The reaction mixture was diluted with EtOAc (100 ml), and washed with 0.5 M aq. HCl (2 x 40 ml), 5% aq. $NaHCO_3$ (2 x 40 ml) and brine (30 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (3:1, 1:1 hexanes/EtOAc) to afford **252** as a white foam (Yield: 16.5 g, 48%).

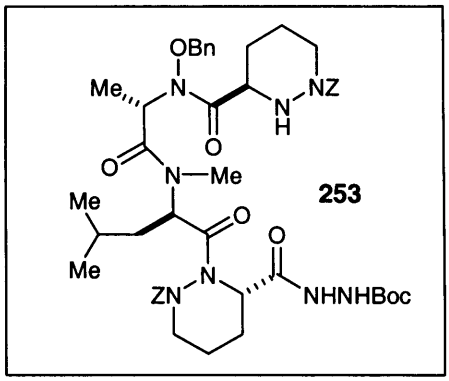
Infra-Red (KBr) (cm^{-1}): 3302 (m), 2957 (m), 1709 (s), 1452 (m), 1413 (m), 1364 (m), 1292 (m), 1250 (m), 1195 (m), 1159 (m), 1049 (w), 1000 (w), 912 (w), 734 (s), 699 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 9.50 (br m), 8.90-8.50 (br m), 7.80-7.0 (br m), 6.20 (br m), 5.60-4.70 (br m), 4.50-3.70 (br m), 3.20-2.80 (br m), 2.30-2.0 (br m), 1.80 (br m), 1.45 (br m), 1.0-0.65 (br m)

125 MHz ^{13}C NMR ($CDCl_3$): δ 172.8, 171.0, 169.9, 156.2, 155.0, 154.6, 144.3, 143.6, 141.7, 141.6, 137.0, 136.8, 135.7, 134.8, 130.6, 129.5, 129.0, 128.9, 128.7, 128.4, 128.2, 128.2, 127.5, 125.7, 125.5, 120.4, 81.7, 81.1, 79.2, 70.0, 69.1, 68.1, 67.9, 59.4, 53.0, 52.1, 50.3, 47.5, 47.3, 45.7, 44.5, 38.2, 32.3, 30.7, 30.1, 29.8, 28.5, 25.5, 24.5, 24.3, 24.2, 23.6, 23.4, 23.1, 21.7, 20.4, 19.5, 19.0, 14.5

FAB HRMS: Calcd. for $C_{63}H_{74}O_{13}N_8Na$ $(M+Na)^+$: 1173.5273; Found: 1173.5231

Optical Rotation: $[\alpha]_D$ = -45.2 ^{0} (*c* 0.15, CH_2Cl_2)



253

To tetrapeptide **252** (40 g, 34.8 mmol) in MeCN (300 ml) was added Et_2NH (140 ml, 1.4 mol) and stirred at RT for 15 min under N_2. The reaction mixture was diluted with EtOAc and concentrated *in vacuo* and the product purified by SiO_2 flash chromatography (4:1, 1:1 hexanes/EtOAc) to afford **253** as a white solid (Yield: 24 g, 74%).

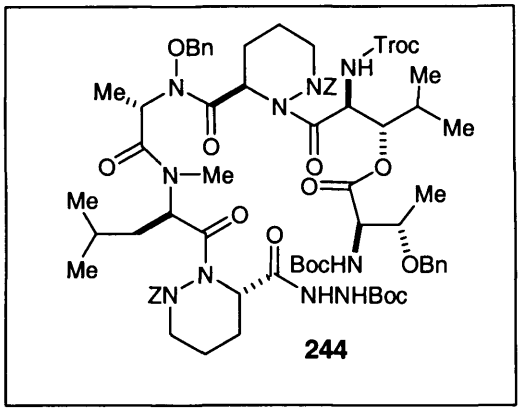
500 MHz ^{1}H NMR ($CDCl_3$): δ 9.5 (br m), 8.95 (br m), 7.40-7.10 (br m), 6.20 (br m), 5.6 (br m), 5.35 (br m), 5.20-4.90 (br m), 4.80 (br m), 4.20 (br m), 3.85 (br m), 3.15 (br m), 3.0 (br m), 2.85 (br m), 2.25 (br m), 2.0-1.65 (br m), 1.55-1.30 (br m), 1.0-0.70 (br m)

125 MHz ^{13}C NMR ($CDCl_3$): δ 174.3, 156.3, 155.1, 137.1, 135.8, 134.8, 134.6, 129.8, 129.6, 129.4, 129.2, 129.1, 129.0, 128.9, 128.9, 128.5, 81.6, 79.5, 70.0, 69.4, 68.0, 67.8, 57.2, 53.5, 52.4, 45.5, 38.3, 32.3, 32.1, 31.1, 30.1, 28.5, 27.4, 25.5, 23.7, 23.1, 22.0, 20.4, 14.5

Infra-Red (KBr) (cm^{-1}): 3287.5 (m), 2955 (s), 1730 (s), 1659 (s), 1454 (m), 1402 (m), 1367 (m), 1260 (m), 1157 (m), 1070 (w), 995 (w), 911 (m), 731 (m), 699 (m), 647 (w)

FAB HRMS: Calcd. for $C_{48}H_{65}O_{11}N_8$ $(M+H)^+$: 929.4773; Found: 929.4780

Optical Rotation: $[\alpha]_D$ = -41.3 ^{0} (*c* 0.08, CH_2Cl_2)



244

To crude acid chloride **54** was added a solution of tetrapeptide **253** (7.6 g, 8 mmol) in dry C_6H_6 (75 ml) at RT and under N_2. With stirring and maintaining the N_2 atmosphere, AgCN (1.72 g, 13 mmol) was added in one portion. The reaction vessel was fitted with a reflux condenser, and immersed in an oil bath, pre-heated to 70 ^{0}C, for 8 min, with the apparatus covered with aluminium foil and the system under N_2. The reaction mixture was cooled and diluted with EtOAc (50 ml), filtered through Celite, and the Celite pad washed thoroughly with EtOAc. The filtrate was concentrated *in vacuo* and the product purified by SiO_2 flash chromatography (3:1, 1:1 hexanes/EtOAc) to afford **244** as a white foam (Yield: 9.5 g, 76% 2 steps).

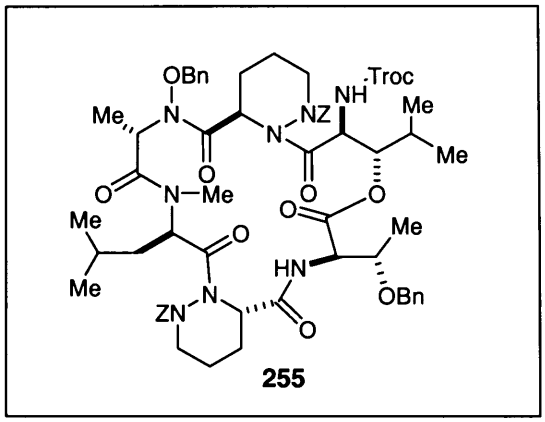
Infra-Red (KBr) (cm^{-1}): 3376 (br m), 2961 (m), 1714 (s), 1660 (s), 1503 (m), 1454 (m), 1392 (m), 1367 (w), 1249 (m), 1161 (m), 1123 (w), 1075 (w), 1041 (w), 1021 (w), 986 (w), 913 (w), 733.1 (w), 699 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 9.5 (br m), 8.80-8.50 (br m), 7.50-7.10 (br m), 6.20 (br m), 5.70 (br m), 5.30-4.90 (br m), 4.65 (d), 4.50 (br m), 4.35 (br m), 4.15 (br m), 3.70 (br m), 3.10 (br m), 2.80 (br m), 2.25 (br m), 2.10-1.90 (br m), 1.75 (br m), 1.60-1.10 (br m), 1.0-0.65 (br m)

125 MHz ^{13}C NMR ($CDCl_3$): δ 172.5, 172.1, 170.5, 156.8, 156.1, 154.9, 153.7, 138.2, 136.3, 135.7, 134.7, 129.7, 129.0, 128.8, 128.7, 128.5, 128.0, 127.9, 126.9, 95.7, 81.7, 81.1, 80.3, 79.3, 75.1, 74.9, 74.7, 70.9, 69.1, 68.7, 59.5, 58.6, 52.9, 52.3, 51.5, 47.6, 46.0, 38.2, 30.5, 30.1, 29.2, 28.8, 28.6, 28.5, 25.5, 24.9, 23.6, 21.6, 20.4 20.2, 17.7, 16.9, 16.6, 14.5

FAB HRMS: Calcd. for $C_{73}H_{97}O_{19}N_{10}Cl_3Na$ $(M+Na)^+$: 1545.5895; Found: 1545.5920

Optical Rotation: $[\alpha]_D$ = -33.3 ^{0} (*c* 0.08, CH_2Cl_2)



255

To hexadepsipeptide **244** (19 g, 12.5 mmol) in dry CH_2Cl_2 (190 ml) at 0 ^{0}C under N_2 was added CF_3CO_2H (190 ml, 2.5 mol) in one portion. The reaction mixture was left to stir at 0 ^{0}C for 2 h, concentrated *in vacuo* and then co-evaporated with PhMe (2x30 ml) to remove the excess CF_3CO_2H. To the resulting residue in THF (140 ml) and H_2O (140 ml) at RT was added NBS (4.45 g, 25 mmol) portionwise over 5 min. The reaction mixture was allowed to stir for 1 h at RT, diluted with EtOAc (100 ml) and washed with brine (2 x 50 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield **254** as a white foam. To a suspension of HATU (14.5 g, 38 mmol) in dry CH_2Cl_2 (4.4 L) at 0 ^{0}C and under N_2 was added dropwise over 3 h a solution of the above crude foam **254** (5 g, 3.8 mmol) and *N*-ethylmorpholine (6.5 ml, 51.3 mmol) in dry CH_2Cl_2 (4.4 L). After the addition the reactants were allowed to warm to RT and stirred for 60 h. The reaction mixture was concentrated *in vacuo* and the residue taken up in EtOAc and washed with 1M aq. HCl (2 x 25 ml), 5% aq. $NaHCO_3$ (2 x 25 ml) and brine (30 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (3:1, 1:1 hexanes/EtOAc) to afford **255** as a white solid (Yield: 1.67 g, 34% 3 steps)

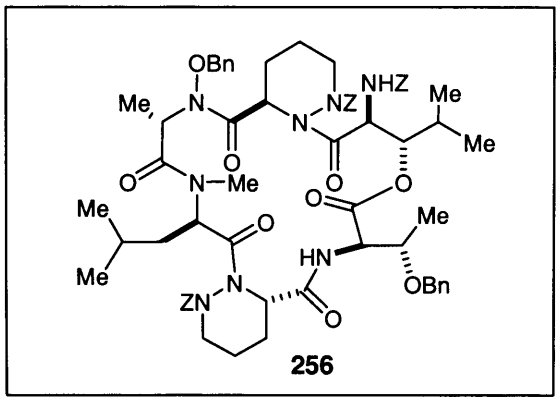
Infra-Red (KBr) (cm^{-1}): 3428 (br m), 2960 (m), 1731 (s), 1659 (s), 1504 (m), 1454 (m), 1384 (m), 1257 (m), 1085 (w), 911 (m), 731 (s), 698 (w)

500 MHz ^{1}H NMR (CD_3OD): δ 7.50-7.10 (br m), 6.50-6.30 (br m), 5.95 (br m), 5.80 (br m), 5.60 (br m), 5.40-4.80 (br m), 4.80-4.30 (br m), 4.30-3.95 (br m), 3.60 (br m), 3.20 (br m), 3.0 (br m), 2.75 (br m), 2.50 (br m), 2.20-1.70 (br m), 1.60-1.30 (br m), 1.30-1.10 (br m), 1.0-0.65 (br m)

125 MHz ^{13}C NMR (CD_3OD): δ 171.8, 169.8, 168.3, 168.1, 167.8, 167.1, 166.9, 156.7, 153.8, 151.9, 151.2, 135.3, 133.4, 133.2, 126.8, 126.7, 126.7, 126.6, 126.5, 126.4, 126.2, 126.1, 126.1, 126.0, 126.0, 125.8, 125.7, 125.7, 125.3, 93.1, 93.0, 77.1, 76.5, 73.5, 72.8, 72.7, 72.6, 69.1, 67.1, 67.1, 54.8, 54.5, 49.2, 48.9, 44.1, 29.3, 27.7, 26.7, 26.6, 22.6, 22.4, 21.0, 20.8, 20.7, 17.5, 17.2, 16.7, 16.4, 14.9, 13.8, 13.5, 13.4, 13.3, 13.0

FAB HRMS: Calcd. for $C_{63}H_{77}O_{15}N_8Cl_3Na$ $(M+Na)^+$: 1313.4472; Found: 1313.4531

Optical Rotation: $[\alpha]_D$ = -24.2 ^{0} (*c* 0.06, CH_2Cl_2)



256

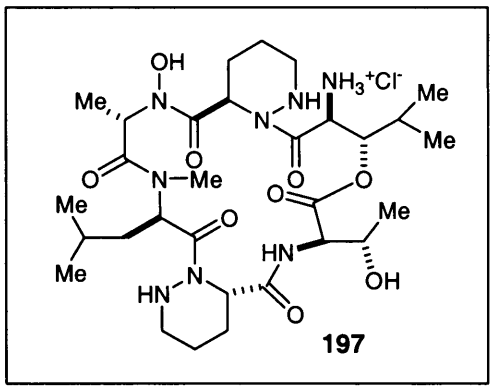
To cyclodepsipeptide **255** (5 g, 3.9 mmol) in a solution of AcOH: H_2O (10:1, 100 ml) at RT was added Zn dust (5 g, 77 mmol). The reaction mixture was allowed to stir vigorously for 25 min, diluted with THF and filtered through Celite. The Celite pad was washed well with THF and the filtrate concentrated *in vacuo*. To the resulting residue was added a solution of Z-Cl (1.7 ml, 11.7 mmol) in CH_2Cl_2 (20 ml) and 10% aq. $NaHCO_3$ (20 ml) simultaneously and dropwise over 10 min. The reaction mixture was left to stir at RT for 1 h, diluted with EtOAc (30 ml) and washed with brine (10 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (2:1 hexanes/EtOAc) to afford **256** as a white solid. (Yield: 3 g, 62% 2 steps).

Infra-Red (KBr) (cm^{-1}): 3424 (m), 3311 (m), 3032 (w), 2959 (m), 1730 (s), 1666 (m), 1499 (m), 1454 (m), 1392 (m), 1348 (m), 1242 (m), 1126 (w), 1083 (w), 1047 (w), 1028 (w), 1000 (w), 910 (m), 732 (m), 698 (m)

500 MHz ^{1}H NMR ($CDCl_3$): δ 7.50-7.0 (br m), 6.40 (br m), 6.0-5.80 (br m), 5.55- 4.75 (br m), 4.70-4.55 (br m), 4.55-4.30 (br m), 4.20 (br m), 4.0-3.70 (br m), 3.20 (br m), 3.10-3.0 (br m), 2.80 (br m), 2.50 (br m), 2.20-1.70 (br m), 1.65-1.35 (br m), 1.20 (br m), 1.0-0.70 (br m)

FAB HRMS: Calcd. for $C_{68}H_{82}O_{15}N_8Na$ $(M+Na)^+$: 1273.5797; Found: 1273.5743

Optical Rotation: $[\alpha]_D$ = +72.5^0 (*c* 0.04, CH_2Cl_2)



197

To cyclodepsipeptide **256** (3 g, 2.4 mmol), in dry methanolic HCl (0.01 M, 240 ml, 2.4 mmol) was added 10% wet Pd/C (2 g) and the system purged with H_2. The reaction mixture was allowed to stir vigorously at RT for 24 h, diluted with MeOH (70 ml) and filtered through Celite. The Celite pad was washed well with MeOH and the filtrate concentrated *in vacuo* to yield **197** as a white solid (Yield: 1.7 g, 100%).

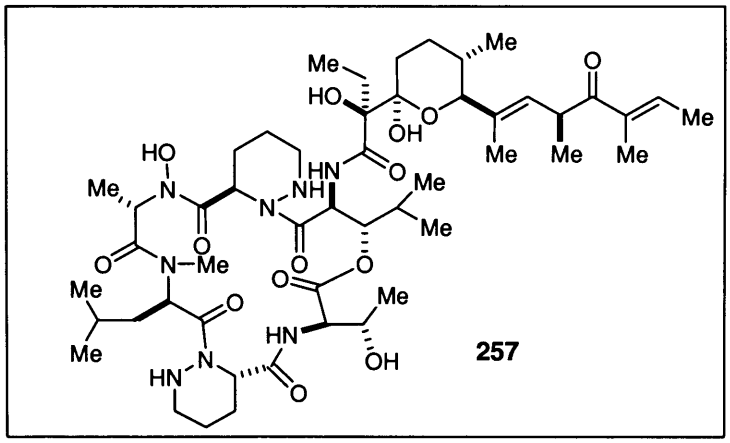
Infra-Red (KBr) (cm^{-1}): 3444 (br s), 2954 (s), 2867 (m), 1747 (m), 1632 (s), 1540 (m), 1442 (m), 1404 (m), 1311 (w), 1256 (m), 1213 (m), 1153 (w), 1104 (w), 1060 (w), 1011 (w), 951 (w), 919 (w), 864 (w), 782 (w), 592 (m)

500 MHz ^{1}H NMR ($CDCl_3$): δ 8.30 (br m), 6.05 (br m), 5.35 (br m), 5.15 (br m), 4.45 (br m), 4.0 (br m), 3.35 (s), 3.30 (s), 3.10 (br m), 2.85 (br m), 2.70 (br m), 2.10 (br m), 1.90 (br m), 1.70 (br m), 1.50 (d, *J* = 7.0 Hz), 1.40 (d, *J* = 6.9 Hz), 1.20 (d, *J* = 6.5 Hz), 1.10 (d, *J* = 7.0 Hz), 1.0 (d, *J* = 6.9 Hz), 0.90 (d, *J* = 6.9 Hz)

125 MHz ^{13}C NMR ($CDCl_3$): δ 174.8, 171.8, 171.3, 170.7, 170.5, 166.9, 78.8, 75.2, 68.9, 68.6, 66.3, 66.1, 57.7, 57.0, 56.7, 49.7, 46.6, 36.3, 26.4, 24.4, 23.1, 21.6, 21.4, 19.8, 19.2, 18.6, 17.6, 17.5, 13.0, 11.0

FAB HRMS: Calcd. for $C_{30}H_{53}O_9N_8$ $(M+H)^+$: 669.3936; Found: 669.3962

Optical Rotation: $[\alpha]_D$ = +36.8 ^{0} (*c* 0.11, MeOH)



To activated ester **47** (86 mg, 0.18 mmol) under N_2 was added cyclodepsipeptide salt **197** (130 mg, 0.18 mmol). The reaction flask was cooled to -78 ^{0}C and a stock solution* of dry Et_3N in dry DMF (0.77 ml, equivalent to 0.0513 ml, 0.37 mmol of Et_3N) was added in one portion. The solution immediately solidified, therefore the vessel was removed from the -78 ^{0}C bath after two min and allowed to warm to RT and left for 15 min. The reaction mixture was diluted with Et_2O (25 ml) and washed successively with 0.5 M aq. HCl (15 ml), sat. aq. $NaHCO_3$ (15 ml) and brine (15 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by preparative TLC using 20:1 CH_2Cl_2:MeOH as the eluant (this was conducted with two batches of the crude product) and subsequently by SiO_2 flash chromatography (2:1 then 0:1,

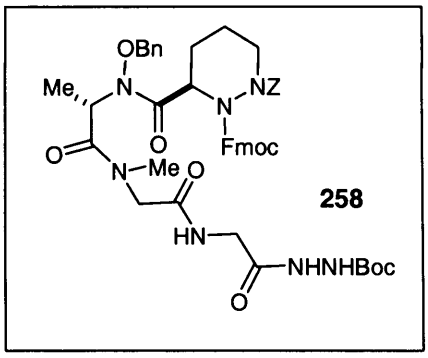
hexanes/EtOAc), to afford **257** as a white solid (Yield: 77 mg, 41%). *(Stock Solution: 1 ml of dry Et_3N in 14 ml of dry DMF).

Infra-Red (KBr) (cm^{-1}): 3408 (br s), 2968 (m), 2932 (m), 2861 (w), 1729 (m), 1663 (s), 1640 (s), 1497 (m), 1467 (m), 1443 (m), 1408 (m), 1384 (m), 1319 (m), 1265 (m), 1212 (m), 1146 (w), 1116 (w), 1063 (m), 1021 (w), 998 (w), 908 (w), 730 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 9.84 (br s, 1H), 8.22 (d, *J* = 10.7 Hz, 1H), 6.71 (dq, *J* = 7.0 Hz, 1.3 Hz, 1H), 6.24 (t, *J* = 7.4 Hz, 1H), 6.30 (br, 1H), 6.16 (d, *J* = 8.4 Hz), 5.60 (dd, *J* = 9.1 Hz, 1.2 Hz, 1H), 5.41 (dd, *J* = 10.8 Hz, 2.2 Hz, 1H), 5.18 (dd, *J* = 5.8 Hz, 1.8 Hz, 1H), 5.11 (q, *J* = 7.0 Hz, 1H), 4.90 (dd, *J* = 7.4 Hz, 3.0 Hz, 1H), 4.89 (t, *J* = 10.7 Hz, 1H), 4.77 (q, *J* = 6.4 Hz), 4.53 (br, 1H), 4.50 (d, *J* = 8.4 Hz, 1H), 4.39 (br d, *J* = 11.9 Hz, 1H), 4.05 (dq, *J* = 9.1 Hz, 7.0 Hz, 1H), 3.95 (d, *J* = 10.3 Hz, 1H), 3.31 (d, *J* = 12.8 Hz, 1H), 3.15 (d, *J* = 13.3 Hz, 1H), 2.98 (s, 3H), 2.95 (m, 1H), 2.91 (s, 1H), 2.60 (m, 1H), 2.56 (d, *J* = 13.1 Hz, 1H), 2.27 (d, *J* = 12.3 Hz, 1H), 2.00 (m, 1H), 1.84 (dd, *J* = 6.9 Hz, 1.1 Hz, 3H), 1.76 (t, *J* = 1.2 Hz, 3H), 1.70-1.60 (m, 10H), 1.56 (d, *J* = 1.3 Hz, 3H), 1.54 (m, 2H), 1.47 (d, *J* = 7.2 Hz, 3H), 1.43 (m, 4H), 1.10 (d, *J* = 6.9 Hz, 3H), 1.04 (d, *J* = 6.5 Hz, 3H), 0.94 (d, *J* = 6.5 Hz, 3H), 0.92 (d, *J* = 6.5 Hz, 3H), 0.82 (t, *J* = 7.4 Hz, 3H), 0.80 (d, *J* = 6.9 Hz, 3H), 0.70 (d, *J* = 6.9 Hz, 3H), 0.68 (d, *J* = 6.6 Hz, 3H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 203.0, 175.3, 174.1, 173.6, 172.2, 170.9, 170.3, 169.5, 137.5, 136.8, 132.8, 129.4, 99.6, 82.2, 78.5, 77.5, 64.8, 56.3, 54.7, 52.2, 51.7, 50.8, 49.5, 47.7, 45.7, 38.2, 36.5, 32.6, 29.4, 29.2, 28.3, 27.3, 25.9, 24.9, 24.4, 24.1, 22.8, 22.7, 21.5, 21.2, 19.5, 19.4, 18.9, 17.6, 14.9, 14.7, 13.4, 12.1, 11.4, 8.2

FAB HRMS: Calcd. for $C_{50}H_{82}O_{14}N_8Na$ $(M+Na)^+$: 1041.5848; Found: 1041.5862



258

To dipeptide **219** (1.0 g, 3.8 mmol) at RT under N_2 was added a solution of dipeptide **52** (2.55 g, 3.8 mmol) in dry CH_2Cl_2 (37 ml). The reaction mixture was cooled to -20 ^{0}C, dry Et_3N (1.07 ml, 7.6 mmol) was added, followed by BOP-Cl (1.16 g, 4.6 mmol) in one portion. The reactants were allowed to stir at -20 ^{0}C for 20 min and then stirred at 0 ^{0}C for 2 h. The reaction mixture was diluted with EtOAc (100 ml), and washed with 0.5 M aq. HCl (2 x 30 ml), 5% aq. $NaHCO_3$ (2 x 30 ml) and brine (30 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (EtOAc) to afford **253** as a white foam (Yield: 2.9 g, 83%).

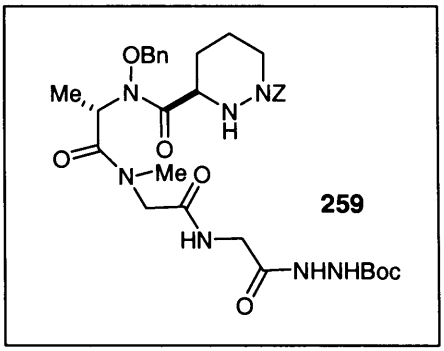
Infra-Red (KBr) (cm^{-1}): 3306 (br m), 3065 (w), 3025 (w), 2975 (w), 2934 (w), 1710(br s), 1554 (w), 1534 (w), 1449 (m), 1409 (m), 1363 (w), 1288 (m), 1253 (m), 1193 (w), 1158 (m), 1093 (w), 1047 (w), 912 (m), 731 (s), 701 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 8.90 (br m), 8.40 (br m), 7.75 (br m), 7.70-7.0 (br m), 6.60-6.45 (br m), 5.50 (br m), 5.30-4.90 (br m), 4.85 (br m), 4.70 (br m), 4.50-4.10 (br m), 4.10-3.90 (br m), 3.75-3.60 (br m), 3.15 (br m), 3.0 (br m), 2.15 (br m),1.75 (br m), 1.40 (br m)

125 MHz ^{13}C NMR ($CDCl_3$): δ 171.5, 171.2, 169.4, 168.9, 168.8, 168.7, 155.9, 155.6, 155.3, 143.7, 143.1, 141.3, 141.3, 136.3, 136.2, 130.2, 129.5, 129.1, 128.6, 128.6, 128.5, 128.3, 128.4, 127.9, 127.8, 127.6, 127.1, 125.2, 120.0, 119.9, 81.4, 81.2, 78.8, 77.6, 68.7, 68.1, 67.8, 67.7, 53.5, 52.9, 47.1, 46.8, 45.2, 44.0, 42.3, 41.9, 41.7, 37.0, 35.7, 33.5, 28.1, 25.4, 24.7, 24.6, 24.1, 23.9, 23.6, 18.6, 14.0, 13.9

FAB HRMS: Calcd. for $C_{48}H_{55}O_{11}N_7Na$ $(M+Na)^+$: 928.38571; Found: 928.38742

Optical Rotation: $[\alpha]_D$ = -44.7 ^{0} (*c* 0.2, CH_2Cl_2)



259

To tetrapeptide **258** (2.9 g, 3.2 mmol) in MeCN (27 ml) was added Et_2NH (13 ml, 0.13 mol) and stirred at RT for 15 min under N_2. The reaction mixture was diluted with EtOAc and concentrated *in vacuo* and the product purified by SiO_2 flash chromatography (1:1, 0:1 hexanes/EtOAc) to afford **259** as a white solid (Yield: 2 g, 91%).

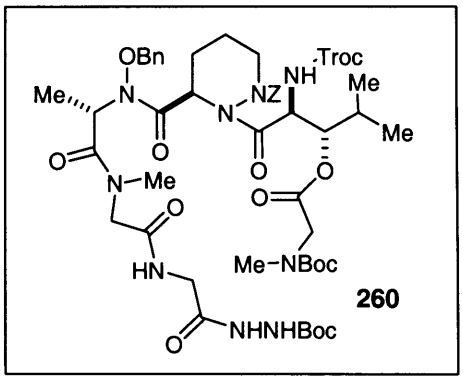
Infra-Red (KBr) (cm^{-1}): 3296 (br m), 3065 (w), 3035 (w), 2975 (w), 2934 (w), 1710 (br s), 1554 (w), 1529 (w), 1499 (w), 1449 (m), 1409 (m), 1363 (m), 1293 (m), 1248 (m), 1193 (m), 1248 (m), 1193 (w), 1158 (m), 1088 (w), 1047 (w), 912 (w), 731 (m)

500 MHz ^{1}H NMR ($CDCl_3$): δ 9.20 (br m), 8.40 (br m), 7.30 (br m), 6.70 (br m), 5.40-4.90 (br m), 4.55 (d), 4.20 (br m), 4.10 (br m), 3.95 (br m), 3.85 (br m), 3.60 (d), 3.30 (s), 3.0 (br m), 1.90 (br m), 1.75 (br m), 1.60 (br m), 1.40 (br m)

125 MHz ^{13}C NMR ($CDCl_3$): δ 172.5, 168.5, 155.8, 155.5, 136.6, 133.9, 129.5, 129.1, 128.8, 128.6, 128.4, 128.2, 128.1, 80.9, 79.4, 67.6, 56.4, 54.8, 53.7, 52.3, 45.0, 41.8, 37.7, 36.4, 28.1, 27.2, 23.2, 14.4, 13.1

FAB HRMS: Calcd. for $C_{33}H_{46}O_9N_7$ $(M+H)^+$: 684.33515; Found: 684.33229

Optical Rotation: $[\alpha]_D$ = +3.0 ^{0} (*c* 0.33, CH_2Cl_2)



To crude acid chloride **220** (1.43 g, 2.7 mmol) was added a solution of tetrapeptide **259** (1.85 g, 2.7 mmol) in dry C_6H_6 (18.5 ml) at RT and under N_2. With stirring and maintaining the N_2 atmosphere AgCN (0.54 g, 4 mmol) was added in one portion. The reaction vessel was fitted with a reflux condenser, and immersed in an oil bath, pre-heated to 80 ^{0}C, for 3 min, with the apparatus covered in aluminium foil and the system under N_2. The reaction mixture was cooled and diluted with EtOAc (50 ml), filtered through Celite, and the Celite pad washed thoroughly with EtOAc. The filtrate was concentrated *in vacuo* and the product purified by SiO_2 flash chromatography (1:1, 0:1 hexanes/EtOAc) to afford **260** as a white foam (Yield: 2.35 g, 75% 2 steps).

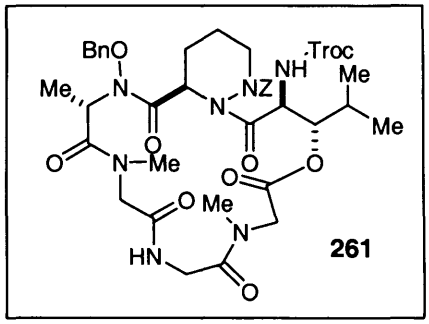
Infra-Red (KBr) (cm^{-1}): 3316 (br m), 2985 (m), 2934 (m), 1740 (s), 1675 (s), 1539 (w), 1504 (w), 1479 (w), 1454 (w), 1399 (m), 1369 (w), 1243 (m), 1193 (w), 1158 (m), 1042 (w), 992 (w), 912 (m), 736 (m)

500 MHz ^{1}H NMR ($CDCl_3$): δ 8.65 (br m), 8.40 (br m), 7.50-7.10 (br m), 7.0-6.50 (br m), 6.05-5.80 (br m), 5.80-5.60 (br m), 5.35-4.95 (br m), 4.90-4.30 (br m), 4.25-3.50 (br m), 3.15 (br m), 2.85 (br m), 2.20-1.70 (br m), 1.45 (br m), 0.95 (br m)

125 MHz ^{13}C NMR ($CDCl_3$): δ 169.1, 155.9, 155.3, 129.2, 128.8, 128.7, 128.4, 128.1, 127.0, 95.5, 80.1, 78.0, 74.6, 52.9, 50.7, 50.1, 41.6, 35.4, 28.3, 28.3, 28.1, 20.1, 16.8

FAB HRMS: Calcd. for $C_{50}H_{70}O_{16}N_9Cl_3Na$ $(M+Na)^+$: 1180.39036; Found: 1180.38454

Optical Rotation: $[\alpha]_D$ = -56.0 ^{0} (*c* 0.16, CH_2Cl_2)



261

To hexadepsipeptide **260** (2.15 g, 1.9 mmol) in dry CH_2Cl_2 (20 ml) at 0 ^{0}C under N_2 was added in one portion CF_3CO_2H (29 ml, 0.38 mol). The reaction mixture was allowed to stir for 2 h, concentrated *in vacuo* and then co-evaporated with PhMe (2 x 20 ml) to remove the excess CF_3CO_2H. To the resulting residue in THF (15 ml) and H_2O (15 ml) at RT was added NBS (0.66 g, 3.7 mmol) portionwise over 5 min. The reaction mixture was left to stir for 2H at RT, diluted with EtOAc (50 ml) and washed with brine (2 x 30 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield a white foam. To a suspension of BOP (1.78 g, 4 mmol) and DMAP (0.61 g, 5 mmol) in dry CH_2Cl_2 (246 ml) at 0 ^{0}C and under N_2 was added dropwise over 4 h a solution of the above solid (0.2 g, 0.2 mmol) in dry CH_2Cl_2 (164 ml). After the addition was complete the reaction mixture was allowed to warm to RT and stirred for 48 h. The reaction mixture was concentrated *in vacuo* and the residue taken up in EtOAc and washed with 1M aq. HCl (2 x 15 ml), 5% aq. $NaHCO_3$ (2 x 15 ml) and brine (15 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (1:3 hexanes/EtOAc) to afford **261** as a white solid (Yield: 146 mg, 74.5% 3 steps).

Infra-Red (KBr) (cm^{-1}): 3416 (m), 3276 (m), 2975 (m), 2944 (m), 1740 (s), 1654 (s), 1539 (m), 1489 (w), 1449 (w), 1404 (w), 1384 (w), 1348 (w), 1303 (w), 1253 (m), 1233 (m), 1198 (w), 1143 (w), 1113 (w), 1088 (w), 1032 (w), 912 (w), 852 (w), 731 (m)

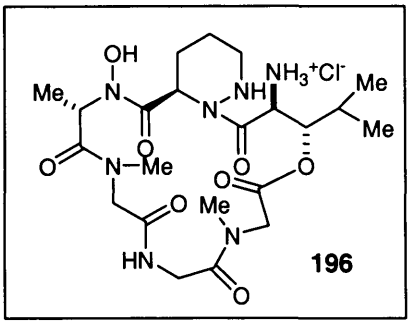
500 MHz ^{1}H NMR ($CDCl_3$): δ 7.40 (br m), 6.90 (d), 6.60 (t), 5.75 (br m), 5.25 (br m), 5.10 (dd), 5.0 (d), 4.90 (br m), 4.70 (d), 4.70 (d), 4.40 (d), 4.30 (d), 3.80 (dd), 3.30 (d), 3.15 (br m), 3.10 (s), 2.80

(s), 2.65 (br m), 2.55 (dd), 1.80 (br m), 2.75 (br m), 2.65 (d), 1.60 (br m), 2.45 (br m), 1.3 (br m), 1.0 (d), 0.9 (d)

125 MHz ^{13}C NMR ($CDCl_3$): δ 170.9, 169.3, 168.9, 168.8, 168.6, 167.8, 155.3, 154.0, 135.7, 132.6, 131.3, 129.5, 128.7, 128.4, 128.3, 128.2, 95.8, 82.1, 79.4, 74.2, 69.0, 54.2, 51.8, 51.2, 49.6, 47.7, 43.0, 40.0, 36.4, 29.0, 25.4, 19.4, 18.9, 18.0, 17.9

FAB HRMS: Calcd. for $C_{40}H_{50}O_{12}N_7Cl_3$ $(M+)^+$: 925.25775; Found: 925.25717

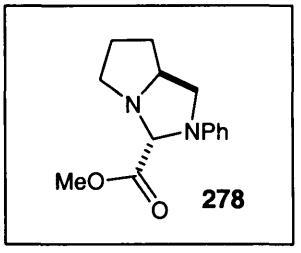
Optical Rotation: $[\alpha]_D$ = -123^0 (*c* 0.67, MeOH)



To cyclodepsipeptide **261** (146 mg, 0.16 mmol) in a solution of AcOH: H_2O (10:1, 3.6 ml) at RT was added Zn dust (670 mg, 10.2 mmol). The reaction mixture was left to stir vigorously for 1 h, diluted with THF and filtered through Celite. The Celite pad was washed well with THF and the filtrate concentrated *in vacuo*. To the resulting residue was added dropwise and simultaneously a solution of Z-Cl (0.1 ml, 0.5 mmol) in CH_2Cl_2 (1 ml) and 10% aq. $NaHCO_3$ (1 ml) over 1 min. The reaction mixture was left to stir at RT for 1 h, diluted with EtOAc (20 ml) and washed with brine (10 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (1:1, 0:1 hexanes/EtOAc) to afford a white solid. (Yield: 76.4 mg, 55% 2 steps). To the white solid obtained (76.4 mg, 0.09 mmol), in dry methanolic HCl (0.01 M, 8.6 ml, 0.086 mmol) was added 10% wet Pd/C (50 mg) and the system purged with H_2. The reaction mixture was left to stir vigorously at RT for 24 h, diluted with MeOH (70 ml) and filtered through Celite. The Celite pad was washed well with MeOH and the filtrate concentrated *in vacuo* to yield **196** as a white solid (Yield: 49 mg, 100%).

500 MHz ^{1}H NMR (CD_3OD): δ 5.37 (q, *J* = 6.3 Hz, 1H), 5.29 (br d, *J* = 2.7 Hz, 1H), 5.08 (br d, *J* = 9.8 Hz, 1H), 5.08 (d, *J* = 17.9 Hz, 1H), 5.08 (1H), 4.47(d, *J* = 17.4 Hz, 1H), 4.04 (d, *J* = 17.2 Hz, 1H), 3.92 (d, *J* = 4.5 Hz, 1H), 3.89 (d, *J* = 4.2 Hz, 1H), 3.76 (d, *J* = 17.6 Hz, 1H), 3.15 (s, 3H), 3.12 (m, 1H), 2.87 (s, 3H), 2.72 (m, 1H), 2.1(d, *J* = 13.7 Hz, 1H), 2.0 (m, 1H), 1.84 (m, 1H), 1.64 (d, *J* = 13.7 Hz, 1H), 1.54 (m, 1H), 1.33 (d, *J* = 6.5 Hz, 3H), 1.17 (d, *J* = 6.5 Hz, 3H), 0.92 (d, *J* = 6.5 Hz, 3H)

125 MHz ^{13}C NMR (CD_3OD): δ 173.9, 172.5, 172.4, 170.5, 170.1, 169.4, 78.6, 54.4, 53.3, 52.8, 51.5, 50.6, 47.7, 42.9, 37.5, 35.1, 30.6, 24.2, 21.9, 19.4(x2), 14.9

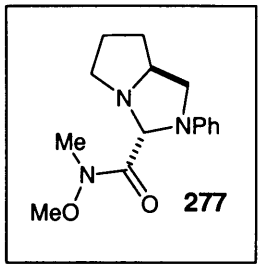


278

To a stirred solution of diamine **266** (12 g, 68 mmol) in dry toluene (67 ml) at RT was added freshly distilled methyl glyoxylate (7.1 g, 80 mmol) dropwise over 2 min. The reaction mixture was stirred at RT for 15 min then concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (Et_2O) to afford **278** as a yellow oil. (Yield: 16.3 g, 97%)

500 MHz ^{1}H NMR ($CDCl_3$): δ In agreement with data from Ref.121

125 MHz ^{13}C NMR ($CDCl_3$): δ In agreement with data from Ref.121

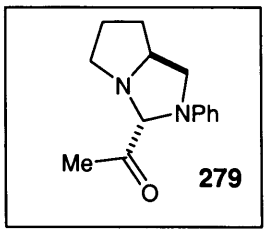


277

To a solution of *N,O*-dimethylhydroxylamine hydrochloride (8.98 g, 92 mmol) in dry THF (300 ml) at 0 ^{0}C and under N_2 was added cautiously Me_3Al (45 ml, 2.0 M soln. in hexanes, 91 mmol). The reaction mixture was allowed to warm to RT over 1 h and then a solution of aminal **278** (15 g, 61 mmol) in dry THF (150 ml) was added over 5 min. The reaction was allowed to stir for 60h. The reaction mixture was cooled to 0 ^{0}C and quenched cautiously with sat.aq. NH_4Cl (150 ml) and then extracted with EtOAc (3 x 250 ml). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (1:1 hexanes/EtOAc, 15:1 EtOAc/MeOH) to afford **277** as a yellow oil. (Yield: 12.4 g, 74%)

500 MHz ^{1}H NMR ($CDCl_3$): In agreement with data from Ref.121

125 MHz ^{13}C NMR ($CDCl_3$): In agreement with data from Ref.121

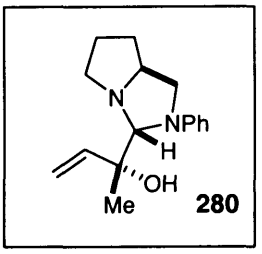


279

To **277** (7.89 g, 29 mmol) in dry THF (395 ml) at -78 ^{0}C and under N_2 was added MeMgBr (17.8 ml, 3.0 M soln. in Et_2O, 50 mmol) over 5 min. The reaction mixture was allowed to stir for 1 h at -78 ^{0}C and then quenched with sat. aq. NH_4Cl (20 ml) and allowed to warm to RT. The organic layer was separated and aqueous layer extracted with Et_2O (2 x 20 ml). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (Et_2O) to afford **279** as a clear oil. (Yield: 5.9 g, 88%)

500 MHz ^{1}H NMR ($CDCl_3$): In agreement with data from Ref.121

125 MHz ^{13}C NMR ($CDCl_3$): In agreement with data from Ref.121



280

To keto-aminal **279** (5.9 g, 25.6 mmol) in dry THF (180 ml) at -78 ^{0}C and under N_2 was added vinylmagnesium bromide (51 ml, 1.0 M soln. in THF, 51.3 mmol) over 10 min. The reactants were allowed to stir for 2 h at -78 ^{0}C, then -33 ^{0}C for 12 h. The reaction mixture was quenched with sat. aq. NH_4Cl (70 ml) and left to warm to RT. The organic layer was removed and the aqueous layer extracted with Et_2O (2 x 100 ml). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (5:1, 1:1 hexanes/Et_2O) to afford **280** as a clear oil (Yield: 5.1 g, 77%)

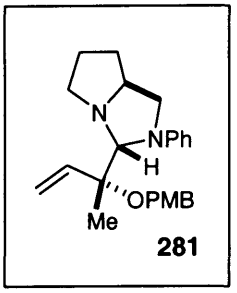
Infra-Red (neat) (cm^{-1}): 3449 (br m), 3091 (w), 3045 (w), 3010 (w), 2964 (m), 2860 (m), 1715 (w), 1599 (s), 1576 (w), 1501 (s), 1455 (w), 1415 (w), 1345 (s), 1311 (m), 1195 (m), 1161 (m), 1103 (m), 1040 (w), 1011 (w), 993 (m), 965 (w), 918 (m), 866 (w), 780 (w), 751 (m), 693 (m)

500 MHz ^{1}H NMR ($CDCl_3$): δ 7.22 (m, 2H), 6.75 (m, 3H), 6.03 (dd, *J* = 17.4 Hz, 10.7 Hz, 1H), 5.35 (dd, *J* = 17.4 Hz, 1.7 Hz, 1H), 5.11 (dd, *J* = 10.7 Hz, 1.7 Hz, 1H), 4.55 (s, 1H), 3.92 (m, 1H), 3.73 (dd, *J* = 9.2 Hz, 7.5 Hz, 1H), 3.22 (m, 1H), 3.15 (dd, *J* = 9.2 Hz, 6.7 Hz, 1H), 3.05 (br s, 1H), 2.61 (m, 1H), 2.14 (m, 1H), 1.80 (m, 2H), 1.66 (m, 2H), 1.30 (s, 3H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 148.2, 142.9, 128.6, 116.9, 113.3, 112.7, 88.7, 77.0, 62.4, 56.4, 56.1, 31.7, 25.1, 24.0

FAB HRMS: Calcd. for $C_{16}H_{23}ON_2$ $(M+H)^+$: 259.18103; Found: 259.17918

Optical Rotation: $[\alpha]_D$ = +60.6 ^{0} (*c* 0.2, CH_2Cl_2)



281

To **280** (0.1 g, 0.39 mmol) in dry DMF (0.5 ml) at 0 ^{0}C was added in one portion 60% NaH dispersion in mineral oil (25 mg, 0.62 mmol) and allowed to stir at 0 ^{0}C for 30 min. PMB-Cl (0.1 ml, 0.50 mmol) was added to the reaction mixture and allowed to warm to RT and stirred for 24 h. The reaction mixture was diluted with H_2O and extracted with Et_2O (2 x 10 ml), the combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (8:1 hexanes/Et_2O) to afford **281** as a clear oil. (Yield: 75 mg, 51%)

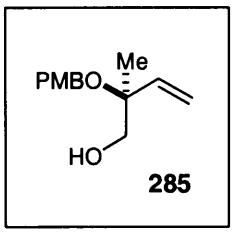
Infra-Red (neat) (cm^{-1}): 2953 (m), 2860 (m), 1593 (m), 1507 (s), 1478 (w), 1470 (w), 1409 (w), 1344 (m), 1300 (w), 1246 (s), 1169 (m), 1147 (m), 1120 (m), 1028 (m), 995 (w), 924 (w), 821 (w), 744 (w), 690 (w)

500 MHz ^{1}H NMR ($CDCl_3$): δ 7.15 (dd, *J* = 7.2 Hz, 1.6 Hz, 2H), 7.10 (d, *J* = 8.7 Hz, 2H), 6.85 (d, *J* = 8.2 Hz, 2H), 6.77 (d, *J* = 8.7 Hz, 2H), 6.67 (t, *J* = 7.2 Hz, 1H), 6.05 (dd, *J* = 17.7 Hz, 10.8 Hz, 1H), 5.30 (dd, *J* = 10.8 Hz, 1.5 Hz, 1H), 5.30 (dd, *J* = 17.7 Hz, 1.5 Hz, 1H), 4.63 (s, 1H), 4.31 (s, 2H), 3.92 (m, 1H), 3.78 (s, 3H), 3.64 (t, *J* = 8.4 Hz, 1H), 3.16 (m, 1H), 3.04 (t, *J* = 8.0 Hz, 1H), 2.49 (q, *J* = 8.4 Hz, 1H), 2.09 (m, 1H), 1.76 (m, 2H), 1.64 (m, 1H), 1.47 (s, 3H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 158.4, 148.8, 140.4, 132.0, 128.4, 128.2, 117.2, 116.0, 113.5, 113.3, 88.8, 83.6, 64.2, 62.0, 56.1, 55.8, 55.2, 31.2, 24.4, 19.0

FAB HRMS: Calcd. for $C_{24}H_{31}O_2N_2$ $(M+H)^+$: 379.23800; Found: 379.23647

Optical Rotation: $[\alpha]_D$ = +12.1 ^{0} (*c* 0.46, CH_2Cl_2)

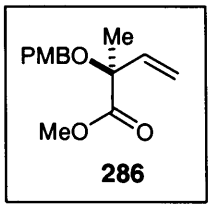


285

A reaction vessel was charged with $(dba)_3Pd_2$-$CHCl_3$ (123 mg, 0.12 mmol), (1R, 2R)-bis-*N*-*N'*-[2-(diphenylphosphino)benzoyl]-diaminocyclohexane **283** (246 mg, 0.36 mmol) and PMB-OH (1.48 ml, 11.9 mmol). The reaction vessel was placed under reduced pressure and refilled with N_2 (x5). At RT and under N_2 was added dry and degassed CH_2Cl_2 (110 ml). The ensuing purple mixture was stirred at RT for 15 min upon which a brown/orange colour forms. Et_3B (0.12 ml, 1.0 M soln. in THF, 0.12 mmol) and isoprene monoepoxide **282** (1.17 ml, 11.9 mmol) were added to the reaction mixture and the colour changed to olive. The reaction was left to stir for 3 h and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (4:1 hexanes/Et_2O) to afford **285** as a clear oil. (Yield: 2.5 g, 95%)

500 MHz ^{1}H NMR ($CDCl_3$): In agreement with data from Ref.125

125 MHz ^{13}C NMR ($CDCl_3$): In agreement with data from Ref.125



286

To dried 4A molecular sieves at RT and under N_2 was added a solution of alcohol **285** (35 g, 0.16 mol) in dry CH_2Cl_2 (500 ml). To the reaction mixture was added *N*-methylmorpholine oxide (37 g, 0.32 mol) and tetrapropyl ammonium perruthenate (0.56 g, 1.6 mmol) and stirred at RT for 8 h. The reaction mixture was filtered through Celite and the filtrate concentrated *in vacuo*. The product was passed through a SiO_2 filter column (Et_2O). To the crude aldehyde in *t*-BuOH (200 ml) and 2-methyl-2-butene (100 ml) at RT was added in 3 portions over 15 min a solution of $NaClO_2$ (43 g, 0.47 mol), NaH_2PO_4 (57 g, 0.47 mol) in H_2O (250 ml). The reaction mixture was

stirred at RT for 1 h, diluted with sat. aq. NH_4Cl and extracted with EtOAc (3 x 200 ml). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. To the resulting crude acid in dry DMF (300 ml) at RT and under N_2 was added K_2CO_3 (65 g, 0.47 mol), and MeI (74 ml, 1.2 mmol). The reactants were allowed to stir for 1h and quenched with H_2O, extracted with Et_2O (2 x 250 ml). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (15:1 hexanes/EtOAc) to afford **286** as an oil. (Yield 23 g, 58% 3 steps)

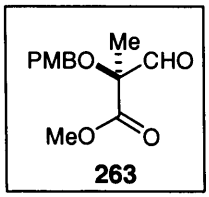
Infra-Red (neat) (cm^{-1}): 2999 (m), 2941 (m), 2972 (w), 2826 (w), 1790 (w), 1732 (s), 1640 (w), 1611 (s), 1582 (w), 1513 (s), 1461 (m), 1409 (w), 1380 (m), 1299 (m), 1247 (s), 1201 (m), 1172 (m), 1115 (s), 1028 (s), 982 (w), 930 (m), 826 (m), 728 (m)

500 MHz ^{1}H NMR ($CDCl_3$): δ 7.31 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 6.08 (dd, J = 17.6 Hz, 10.8 Hz, 1H), 5.44 (d, J = 17.6 Hz, 1H), 5.31 (d, J = 10.8 Hz, 1H), 4.45 (2d, AB system, J = 10.5 Hz, 2H), 3.79 (s, 3H), 3.77 (s, 3H), 1.6 (s, 3H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 173.4, 159.1, 138.2, 130.6, 129.1, 116.5, 113.7, 80.6, 66.7, 55.2, 52.3, 23.4

FAB HRMS: Calcd. for $C_{14}H_{18}O_4$ $(M)^+$: 250.11996; Found: 250.11998

Optical Rotation: $[\alpha]_D$ = -20.0 ^{0} (*c* 0.42, CH_2Cl_2)



263

To methyl ester **286** (5 g, 19.8 mmol) in dry CH_2Cl_2 (190 ml) at RT and under N_2 was added dry MeOH (10 ml). The reaction mixture was cooled to -78 ^{0}C and O_2 was bubbled through for 2 min. O_3 in O_2 was then bubbled through at -78 ^{0}C for 20 min after which a faint blue colour was apparent. O_2 was then bubbled through for a further 2 min. The reaction mixture was

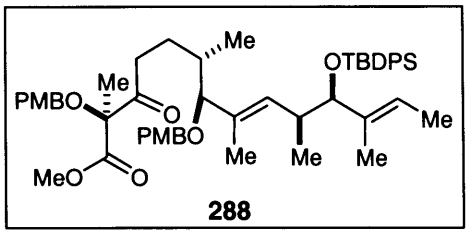
quenched with Me_2S (15 ml, 0.2 mol) and warmed to RT and stirred for 12 h. The reaction mixture was concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (2:1 hexanes/Et_2O) to afford **263** as a clear oil. (Yield: 3.7 g, 74%)

Infra-Red (neat) (cm^{-1}): 3458 (br s), 3001 (w), 2946 (m), 2836 (w), 1755 (s), 1732 (s), 1609 (m), 1581 (w), 1508 (m), 1444 (m), 1380 (w), 1302 (s), 1252 (s), 1174 (m), 1124 (s), 1033 (m), 978 (m)

500 MHz ^{1}H NMR ($CDCl_3$): δ 9.6 (s, 1H), 7.28 (d, *J* = 8.7 Hz, 2H), 6.83 (d, *J* = 8.7 Hz, 2H), 4.48 (2d, AB system, *J* = 10.4 Hz, 2H), 3.73 (s, 3H), 3.72 (s, 3H), 1.54 (s, 3H).

125 MHz ^{13}C NMR ($CDCl_3$): δ 197.3, 169.6, 159.4, 129.6, 129.4, 113.7, 84.8, 67.7, 55.1, 52.6, 18.2

Optical Rotation: $[\alpha]_D$ = +7.5 ^{0} (*c* 0.2, CH_2Cl_2)



288

To sulfone **68** (1 g, 1.35 mmol) in dry THF (4 ml) at -78 ^{0}C and under N_2 was added *n*-BuLi (0.6 ml, 2.5 M soln. in hexanes, 1.47 mmol) dropwise over 2 min. The orange solution was left to stir at -78 ^{0}C for 30 min. The reaction mixture was cannulated into a reaction vessel containing aldehyde **263** (0.34 g, 1.35 mmol) in dry THF (4 ml) dropwise over 5 min at -78 ^{0}C. The reaction mixture was allowed to warm to RT, quenched with H_2O/EtOAc and the organic layer removed. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (5:1, 2:1 hexanes/EtOAc) to afford **287** as a clear oil (Yield: 1.1 g, 84%). To dry CH_2Cl_2 (10 ml) at -78 ^{0}C and under N_2 was added DMSO (0.47 ml, 6.7 mmol) followed by $(CF_3CO)_2O$ (0.47 ml, 3.3 mmol) over 2 min, and the reactants were allowed to stir for 30 min at -78 ^{0}C. A solution of **287** (1.1 g, 1.1 mmol) in dry CH_2Cl_2 (8 ml) was added over 3 min to the reaction mixture followed by dry Et_3N (4.5 ml, 30 mmol) over 5 min. The reaction mixture was warmed to RT, diluted with CH_2Cl_2 and washed with brine (2 x 10 ml). The organic layer was dried

over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was dissolved in THF/H_2O (180 ml, 10:1) and heated at reflux (70 ^{0}C). To the hot mixture was added over a period of 30 min Al/Hg. (The Al foil was activated by dipping in 2% aq. soln. of $HgCl_2$ for 10 secs, then washing first in MeOH then Et_2O and then added straight into the reaction mixture). The reaction mixture was cooled and filtered through Celite and the filter pad was washed thoroughly with EtOAc. The filtrate was concentrated *in vacuo*. The product was purified by SiO_2 flash chromatography (10:1 hexanes/EtOAc) to afford **288** as a clear oil. (Yield: 0.9 g, 95% 2 steps)

500 MHz ^{1}H NMR ($CDCl_3$): δ 7.65 (d, *J* = 7.96 Hz, 2H), 7.60 (d, *J* = 7.96 Hz, 2H), 7.40-7.20 (m, 8H), 7.10 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 8.83 Hz, 2H), 6.75 (d, *J* = 8.67 Hz, 2H), 4.95 (q, *J* = 6.86, 6.31 Hz, 1H), 4.80 (d, *J* = 10.0 Hz, 1H), 4.45 (d, *J* = 10.7 Hz, 1H), 4.35 (d, *J* = 10.7 Hz, 1H), 4.15 (d, *J* = 11.4 Hz, 1H), 3.80 (d, *J* = 11.4 Hz, 1H), 3.75 (s, 3H), 3.70 (s, 3H), 3.65 (s, 3H), 3.0 (d, *J* = 8.9 Hz, 1H), 2.70 (m, 1H), 2.60 (m, 2H), 1.95 (m, 1H), 1.50 (4xs, 12H), 1.20 (d, *J* = 7.0 Hz, 3H), 1.0 (s, 9H), 0.90 (d, *J* = 7.0 Hz, 3H), 0.50 (d, *J* = 6.9 Hz, 3H)

125 MHz ^{13}C NMR ($CDCl_3$): δ 207.6, 170.4, 159.2, 158.8, 136.8, 136.2, 136.1, 134.5, 134.2, 133.6, 131.7, 131.0, 129.8, 129.5, 129.3, 129.0, 127.2, 127.0, 122.5, 113.7, 113.6, 89.7, 86.7, 84.5, 68.9, 67.0, 55.2, 55.2, 52.5, 37.6, 34.9, 34.4, 27.1, 26.9, 19.5, 18.6, 18.2, 15.9, 14.0, 12.8, 11.1, 10.9, 10.4

Optical Rotation: $[\alpha]_D$ = +33.3 ^{0} (*c* 0.03, CH_2Cl_2)

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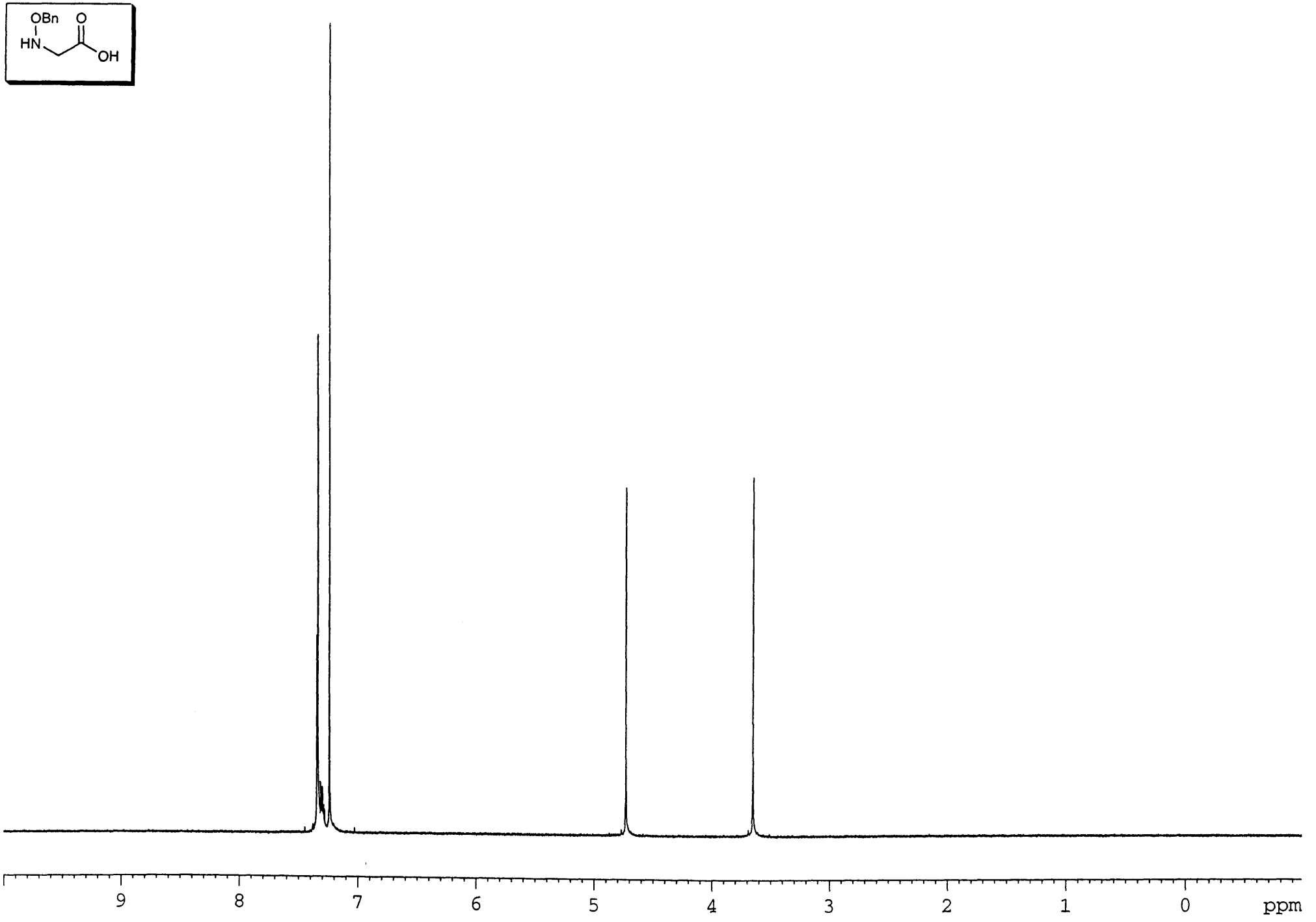
CHAPTER 7 APPENDIX

NMR spectra, FAB LRMS, IR spectra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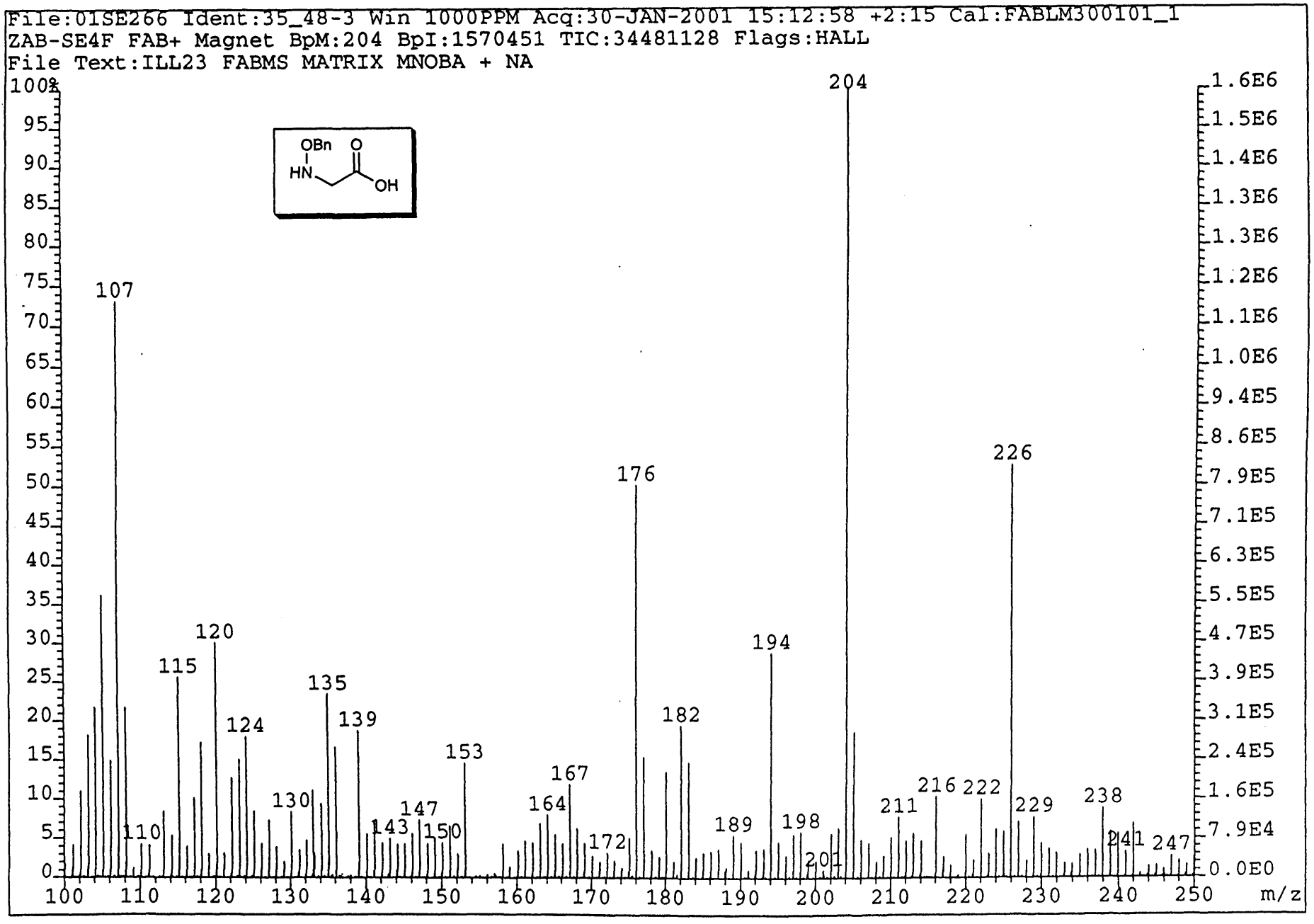
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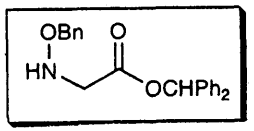
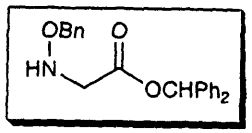
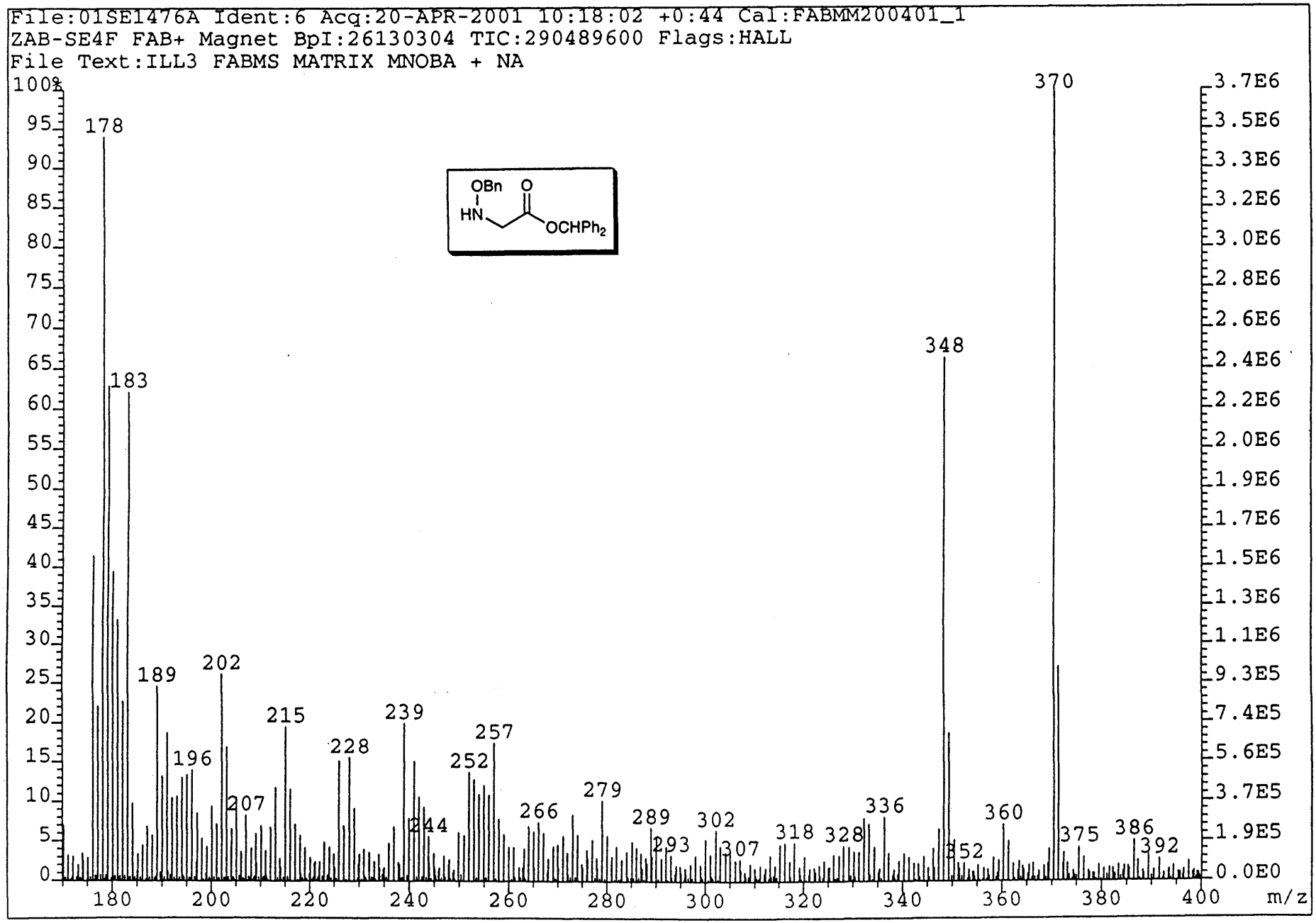
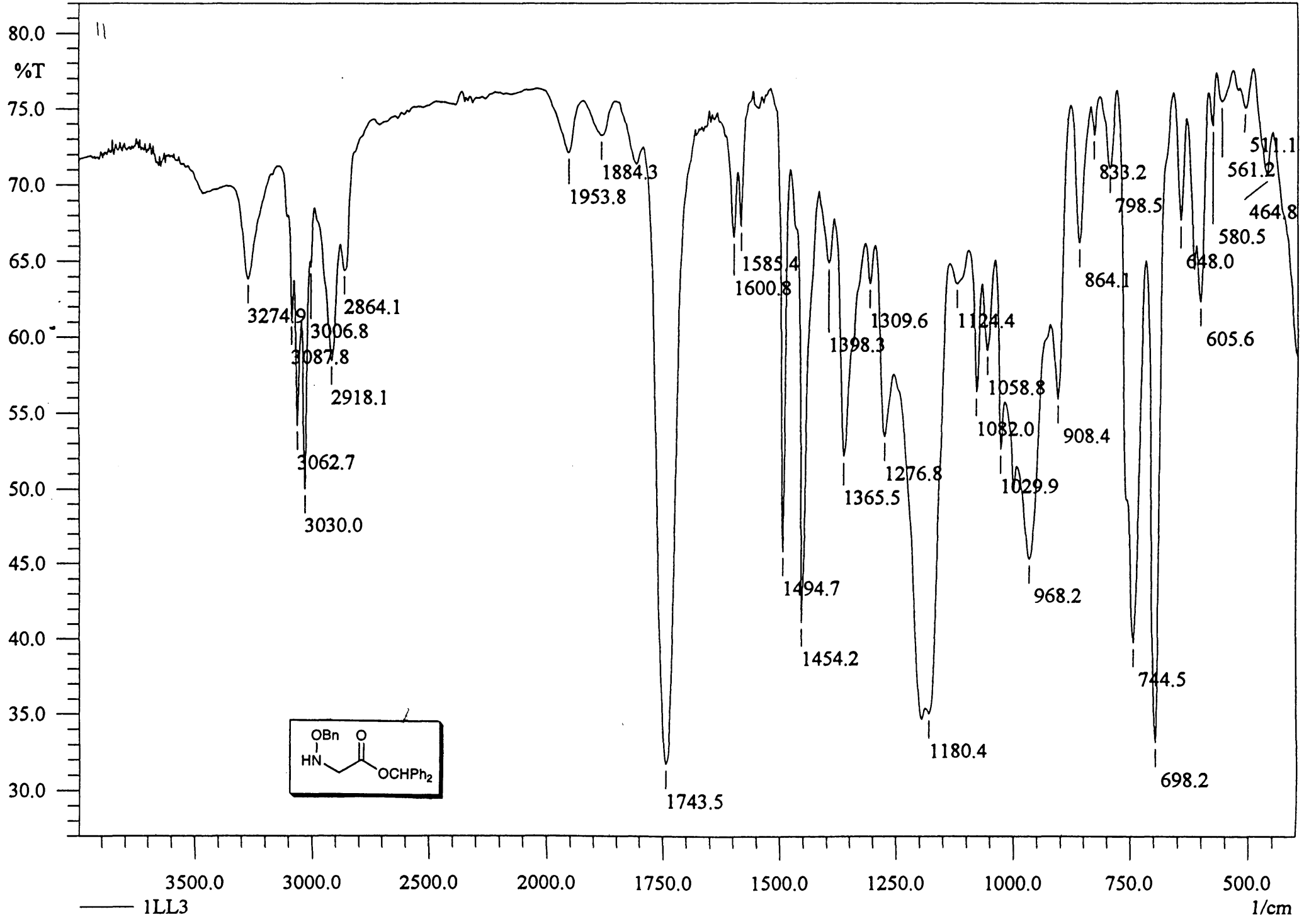


1LL3C

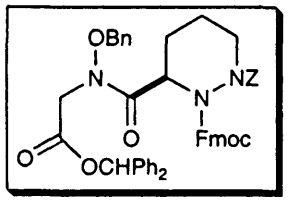

OBn
O
HN
OCHPh2

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

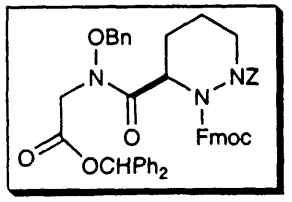

File:01SE1476A Ident:6 Acq:20-APR-2001 10:18:02 +0:44 Cal:FABMM200401_1
ZAB-SE4F FAB+ Magnet BpI:26130304 TIC:290489600 Flags:HALL
File Text:ILL3 FABMS MATRIX MNOBA + NA
OBn
O
HN
OCHPh2
178
183
189
196
202
207
215
228
239
244
252
257
266
279
289
293
302
307
318
328
336
348
352
360
370
375
386
392
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
3.7E6
3.5E6
3.3E6
3.2E6
3.0E6
2.8E6
2.6E6
2.4E6
2.2E6
2.0E6
1.9E6
1.7E6
1.5E6
1.3E6
1.1E6
9.3E5
7.4E5
5.6E5
3.7E5
1.9E5
0.0E0
180
200
220
240
260
280
300
320
340
360
380
400
m/z


80.0
%T
75.0
70.0
65.0
60.0
55.0
50.0
45.0
40.0
35.0
30.0
3274.9
3087.8
3062.7
3030.0
3006.8
2918.1
2864.1
1953.8
1884.3
1743.5
1600.8
1585.4
1494.7
1454.2
1398.3
1365.5
1309.6
1276.8
1180.4
1124.4
1082.0
1058.8
1029.9
968.2
908.4
864.1
833.2
798.5
744.5
698.2
648.0
605.6
580.5
561.2
511.1
464.8
OBn
O
HN
OCHPh2
3500.0
3000.0
2500.0
2000.0
1750.0
1500.0
1250.0
1000.0
750.0
500.0
1LL3
1/cm

1-LL-98

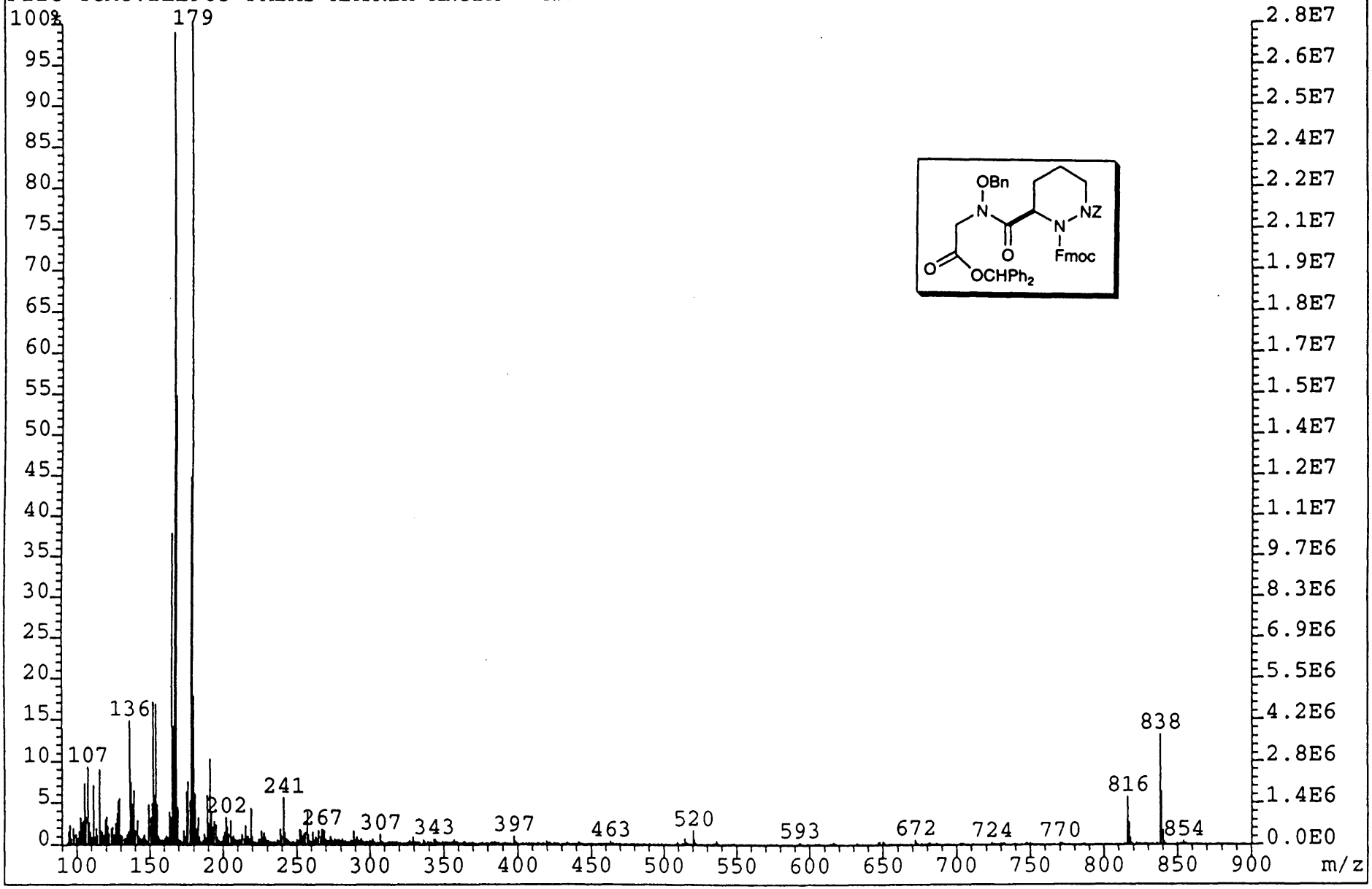


1LL98C

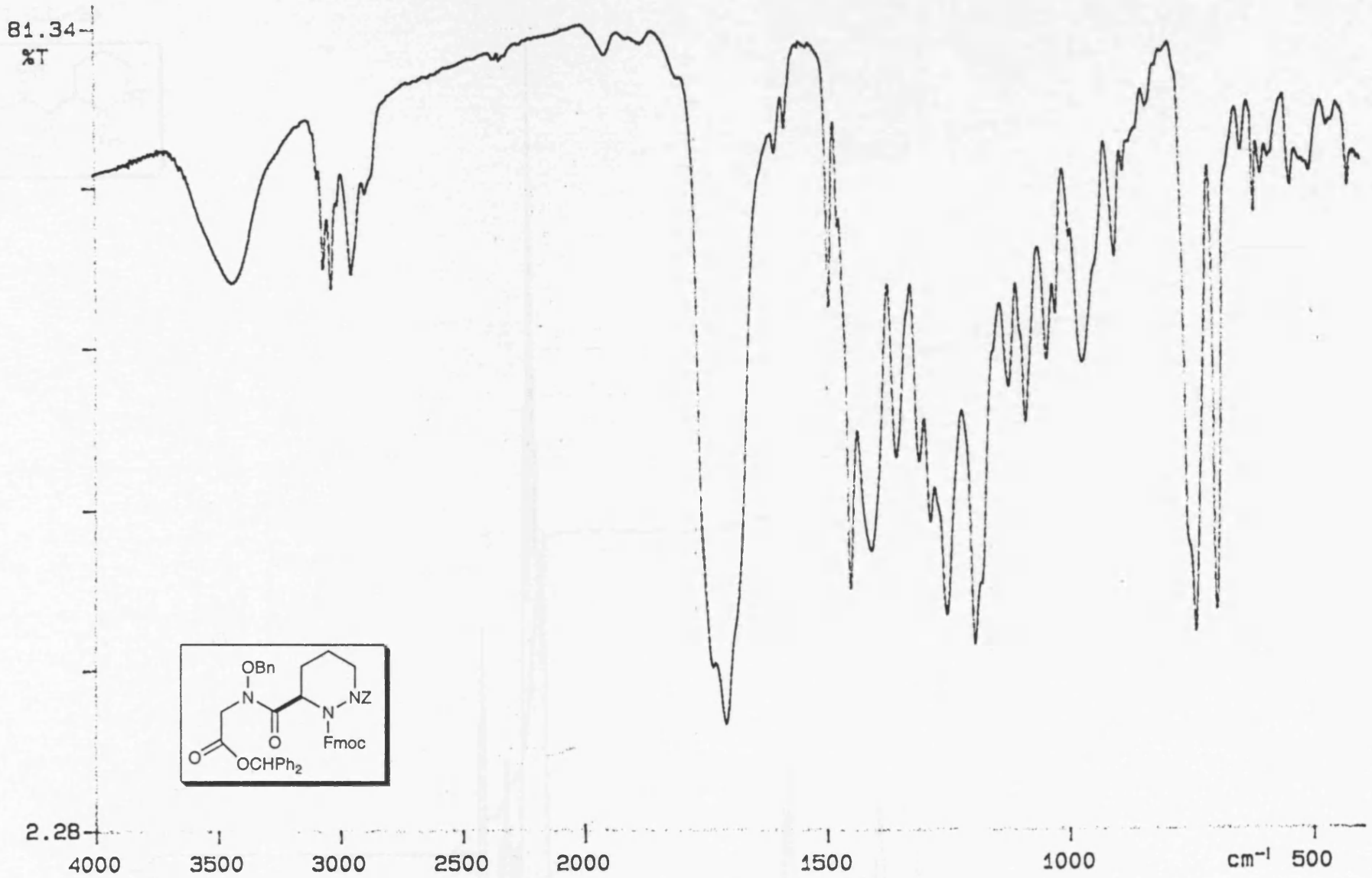

OBn
N
NZ
N
O
Fmoc
O
O
OCHPh2

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

File:01SE1487 Ident:8_19 Win 1000PPM Acq:20-APR-2001 11:39:28 +1:31 Cal:FABMM200401_1
ZAB-SE4F FAB+ Magnet BpM:179 BpI:27692374 TIC:294498400 Flags:HALL
File Text:ILL989 FABMS MATRIX MNOBA + NA

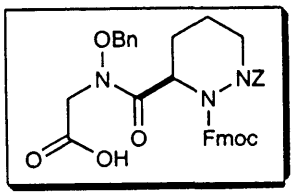


PERKIN ELMER 9

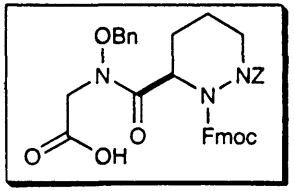


01/02/28 11:29
X: 16 scans, 4.0cm-1

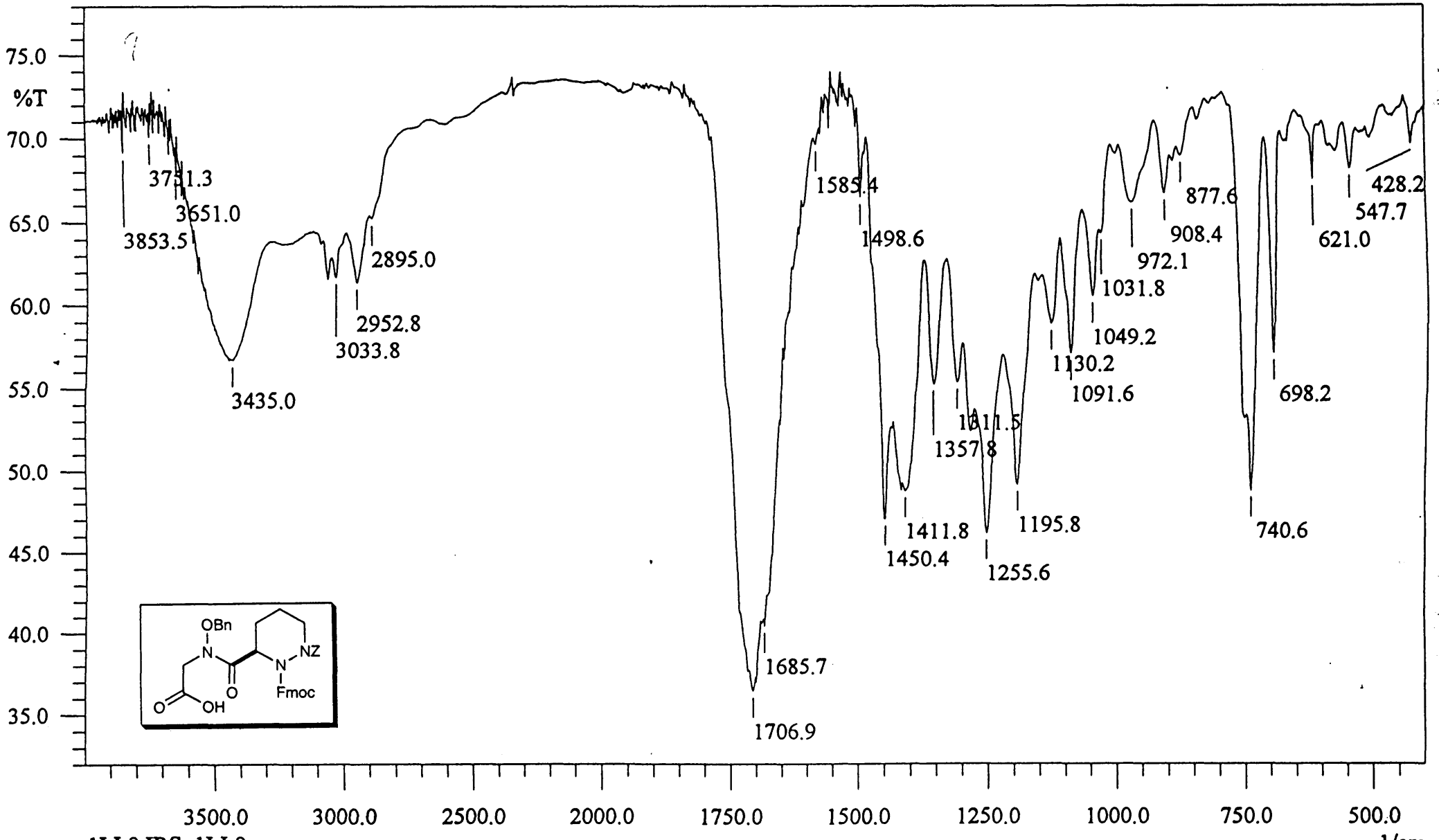
1-LL-99



1-LL-99C

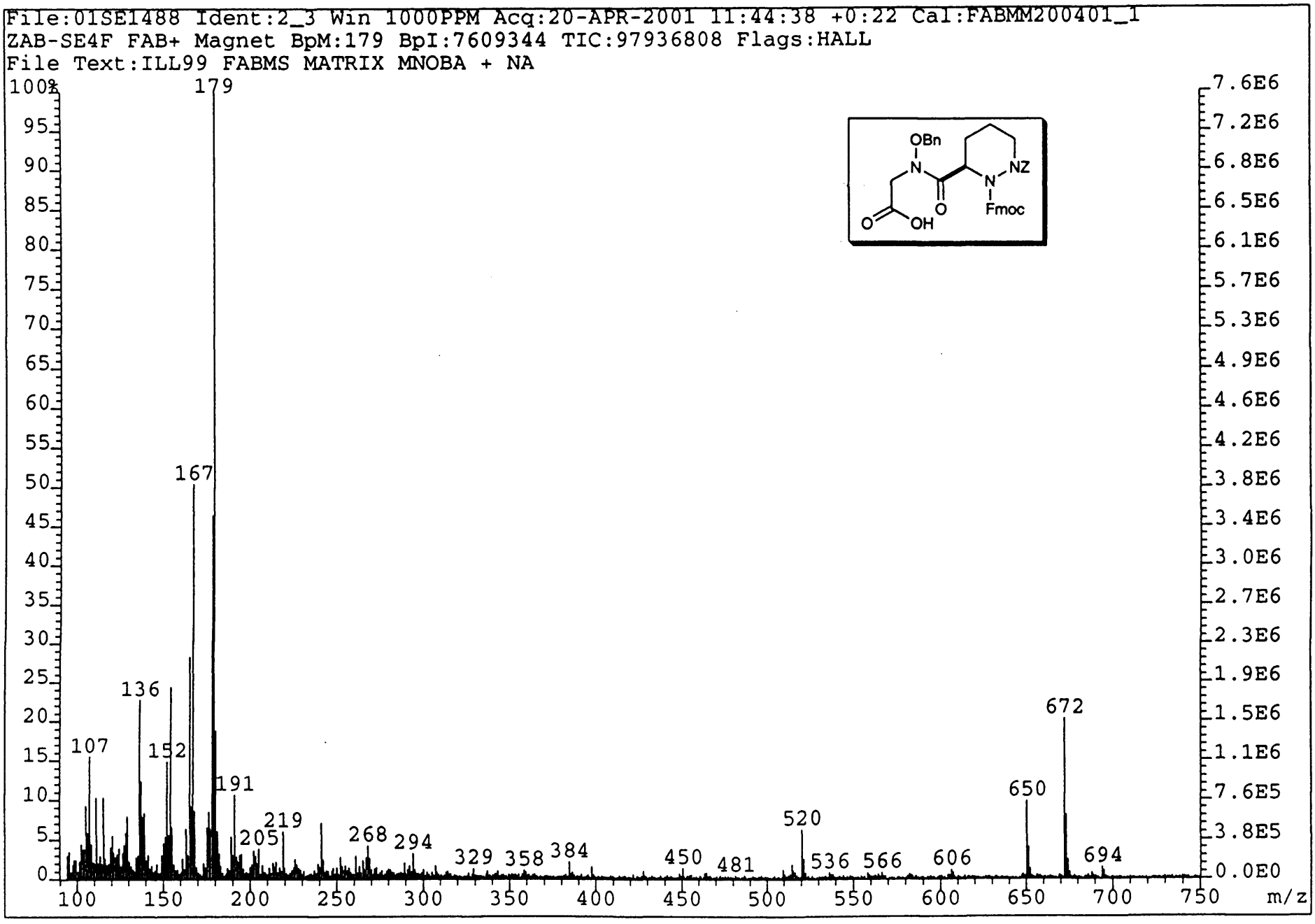

OBn
N
NZ
N
O
Fmoc
O
OH

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm



1LL9.IRS: 1LL9

Date:	06/02/01	Time:	10:32:51	NScans:	20	
Type:	HYPER IR	User:	A20923500085 Shimadz	Detector:	standard	
Abscissa:	1/cm	Ordinate:	%T	Apodization:	Happ	
Min:	401.17	Max:	3998.16	Range:	1/cm	
Ndp:	1866	Data Interval:	1.92868	Resolution:	4.0	
Gain:	auto	Aperture:	auto	Mirror Speed:	2.8(low)	


File:01SE1488 Ident:2_3 Win 1000PPM Acq:20-APR-2001 11:44:38 +0:22 Cal:FABMM200401_1
ZAB-SE4F FAB+ Magnet BpM:179 BpI:7609344 TIC:97936808 Flags:HALL
File Text:ILL99 FABMS MATRIX MNOBA + NA
OBn
N
NZ
N
Fmoc
O
O
OH
179
167
136
107
152
191
219
205
268
294
329
358
384
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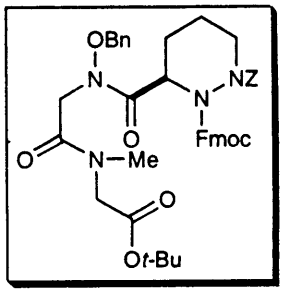
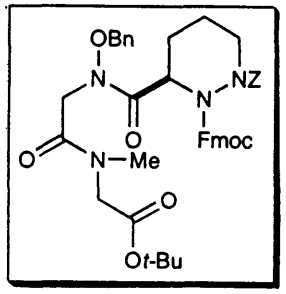
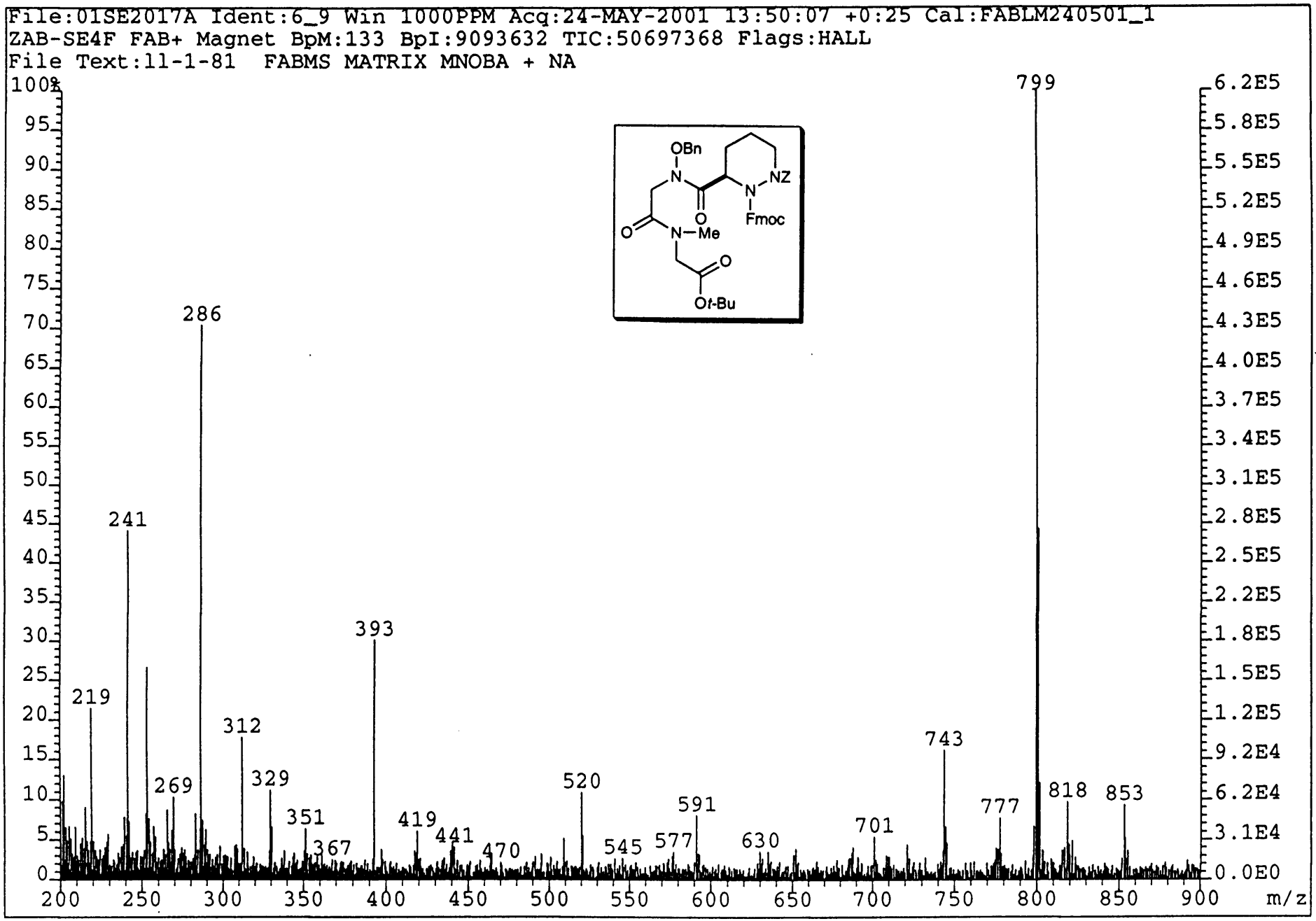
481
520
536
566
606
650
672
694
m/z

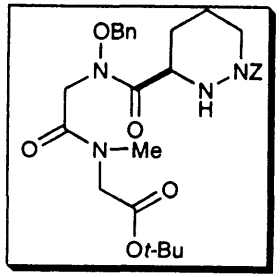

OBn
N
NZ
N
Fmoc
O
O
N–Me
O
Ot-Bu

9
8
7
6
5
4
3
2
1
0
ppm

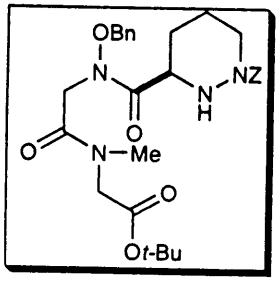

OBn
N
NZ
N
Fmoc
O
O
N–Me
O
O
Ot-Bu

220
200
180
160
140
120
100
80
60
40
20
0
ppm

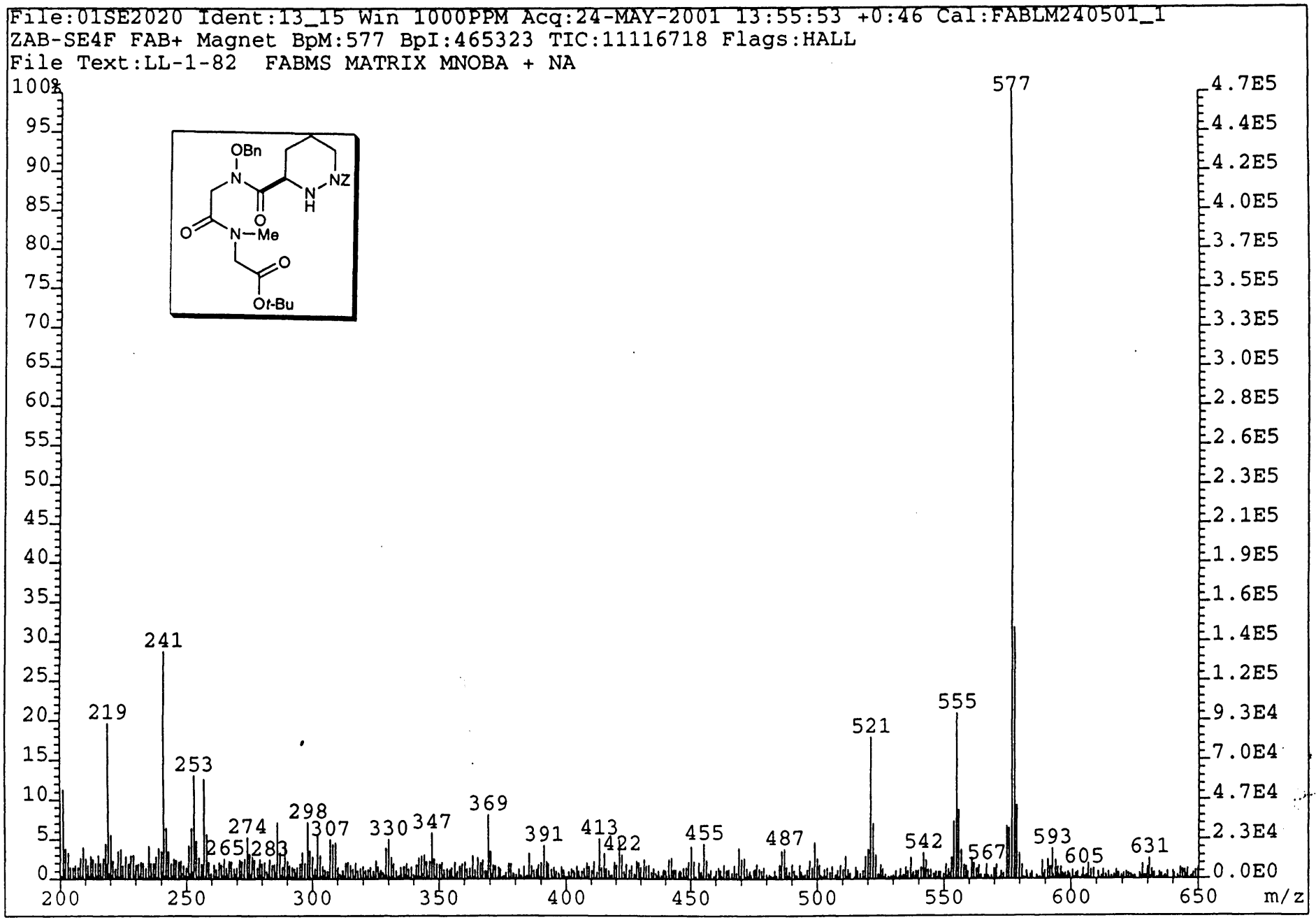

File:01SE2017A Ident:6_9 Win 1000PPM Acq:24-MAY-2001 13:50:07 +0:25 Cal:FABLM240501_1
ZAB-SE4F FAB+ Magnet BpM:133 BpI:9093632 TIC:50697368 Flags:HALL
File Text:11-1-81 FABMS MATRIX MNOBA + NA
OBn
N
NZ
N
Fmoc
O
O
N–Me
O
Ot-Bu
799
286
241
393
219
312
743
329
520
591
269
818
853
777
351
419
441
701
367
470
545
577
630
m/z

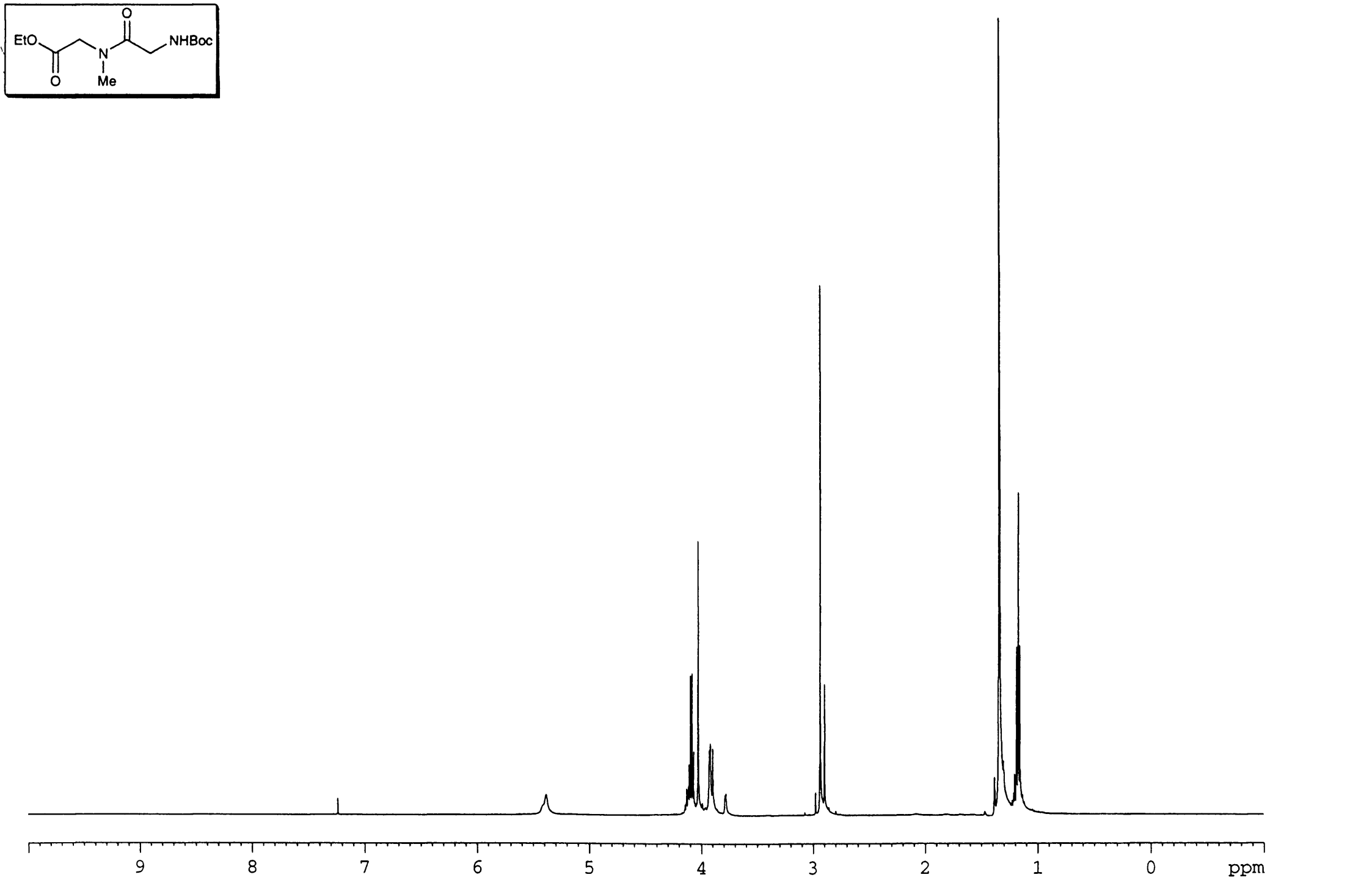

OBn
N
NZ
N
H
O
O
N–Me
O
Ot-Bu

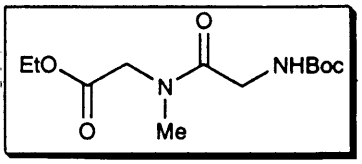
9
8
7
6
5
4
3
2
1
0
ppm


OBn
N
NZ
N
H
O
O
N–Me
O
O
Ot-Bu

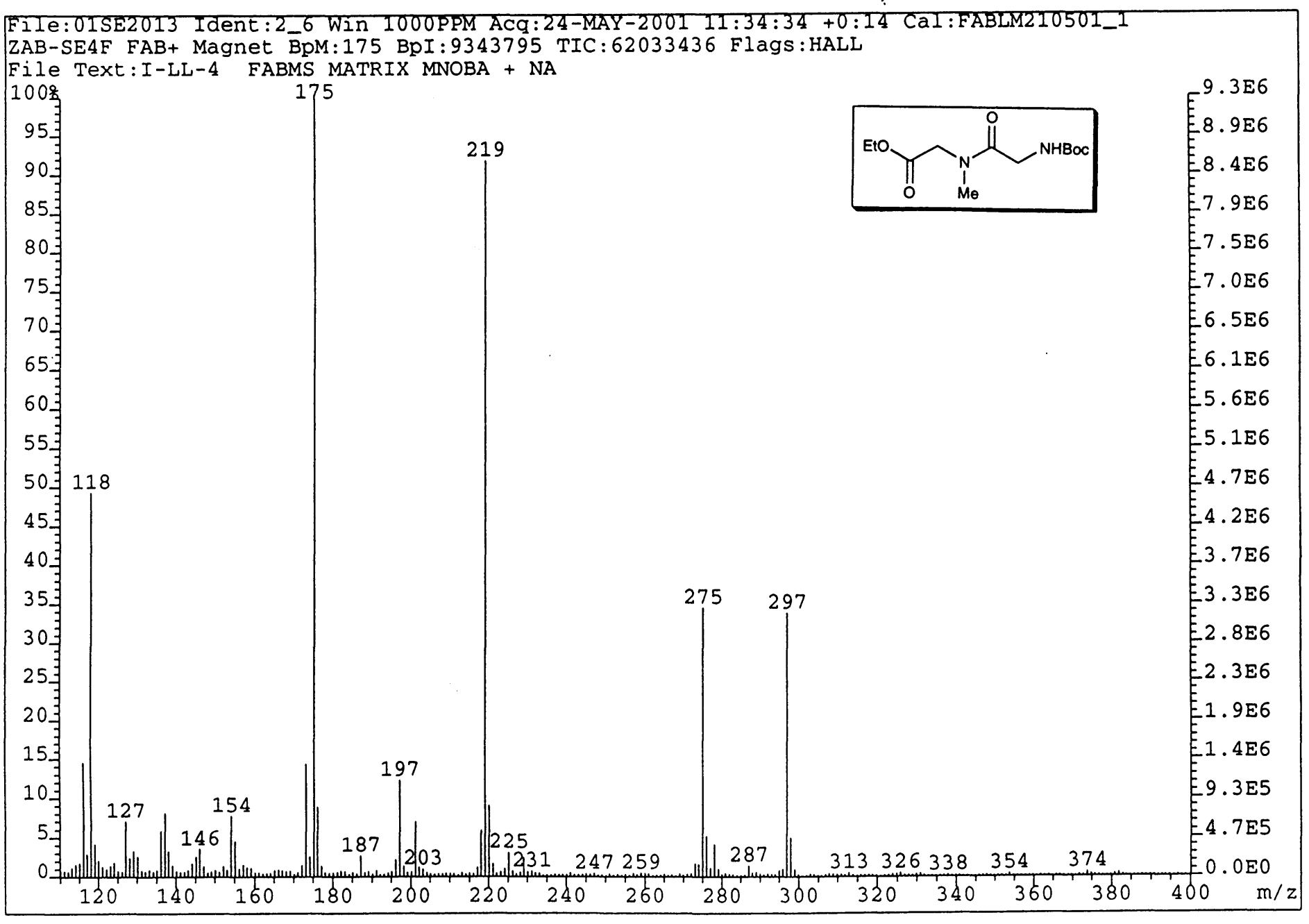
220
200
180
160
140
120
100
80
60
40
20
0
ppm

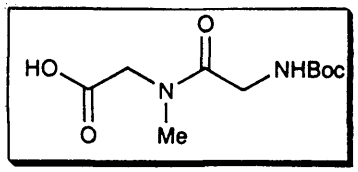

File:01SE2020 Ident:13_15 Win 1000PPM Acq:24-MAY-2001 13:55:53 +0:46 Cal:FABLM240501_1
ZAB-SE4F FAB+ Magnet BpM:577 BpI:465323 TIC:11116718 Flags:HALL
File Text:LL-1-82 FABMS MATRIX MNOBA + NA
OBn
NZ
N
H
O
N–Me
O
O
Ot-Bu
577
241
219
253
555
521
369
298
274
307
330
347
391
413
422
455
487
542
567
593
605
631
265
283
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
4.7E5
4.4E5
4.2E5
4.0E5
3.7E5
3.5E5
3.3E5
3.0E5
2.8E5
2.6E5
2.3E5
2.1E5
1.9E5
1.6E5
1.4E5
1.2E5
9.3E4
7.0E4
4.7E4
2.3E4
0.0E0
200
250
300
350
400
450
500
550
600
650
m/z


EtO
O
N
Me
O
O
NHBoc
9
8
7
6
5
4
3
2
1
0
ppm

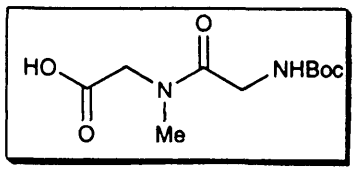

EtO
O
N
Me
O
NHBoc

220
200
180
160
140
120
100
80
60
40
20
0
ppm

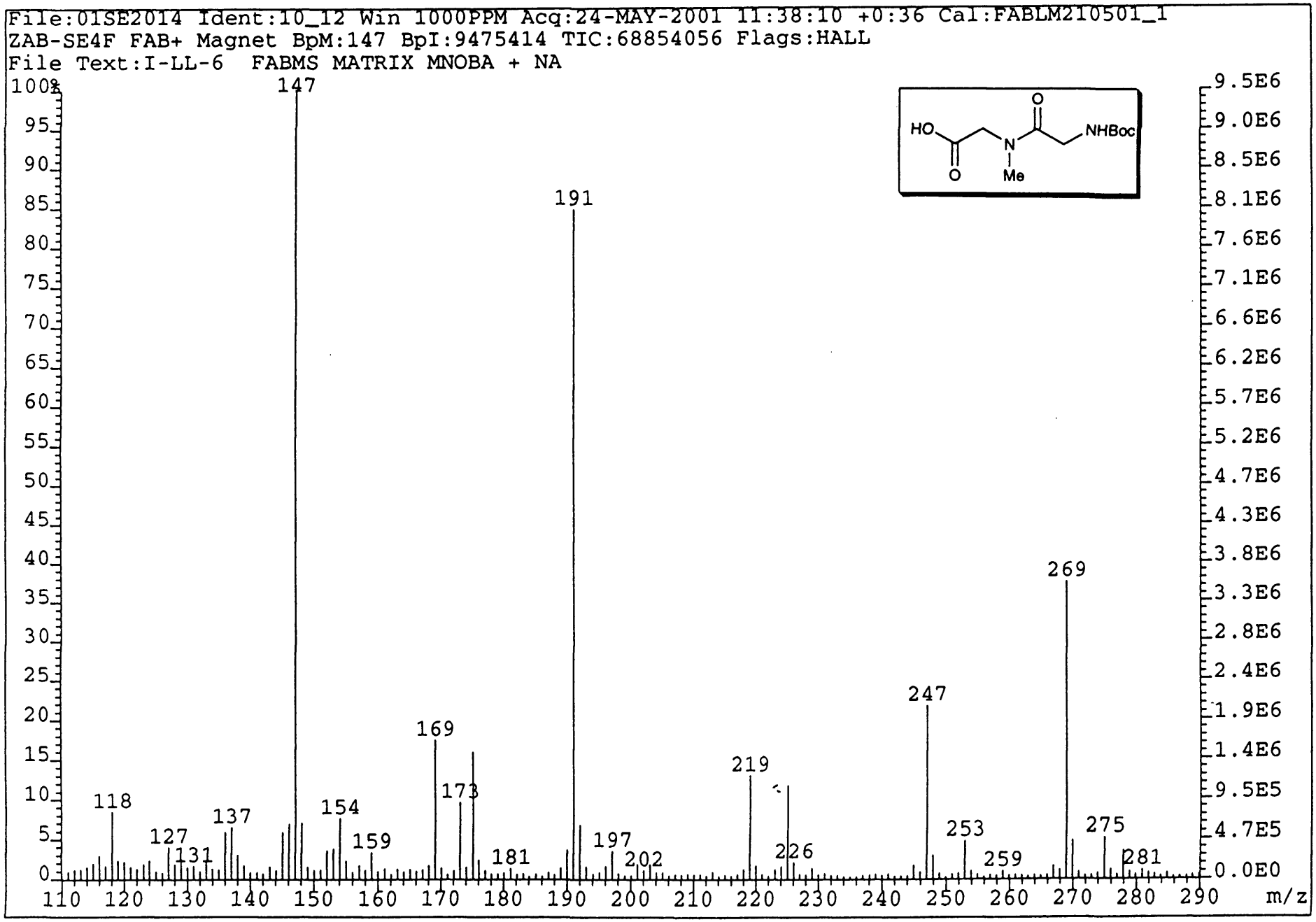

File:01SE2013 Ident:2_6 Win 1000PPM Acq:24-MAY-2001 11:34:34 +0:14 Cal:FABLM210501_1
ZAB-SE4F FAB+ Magnet BpM:175 BpI:9343795 TIC:62033436 Flags:HALL
File Text:I-LL-4 FABMS MATRIX MNOBA + NA
EtO
NHBoc
N
Me
O
O
O
118
127
146
154
175
187
197
203
219
225
231
247
259
275
287
297
313
326
338
354
374
m/z

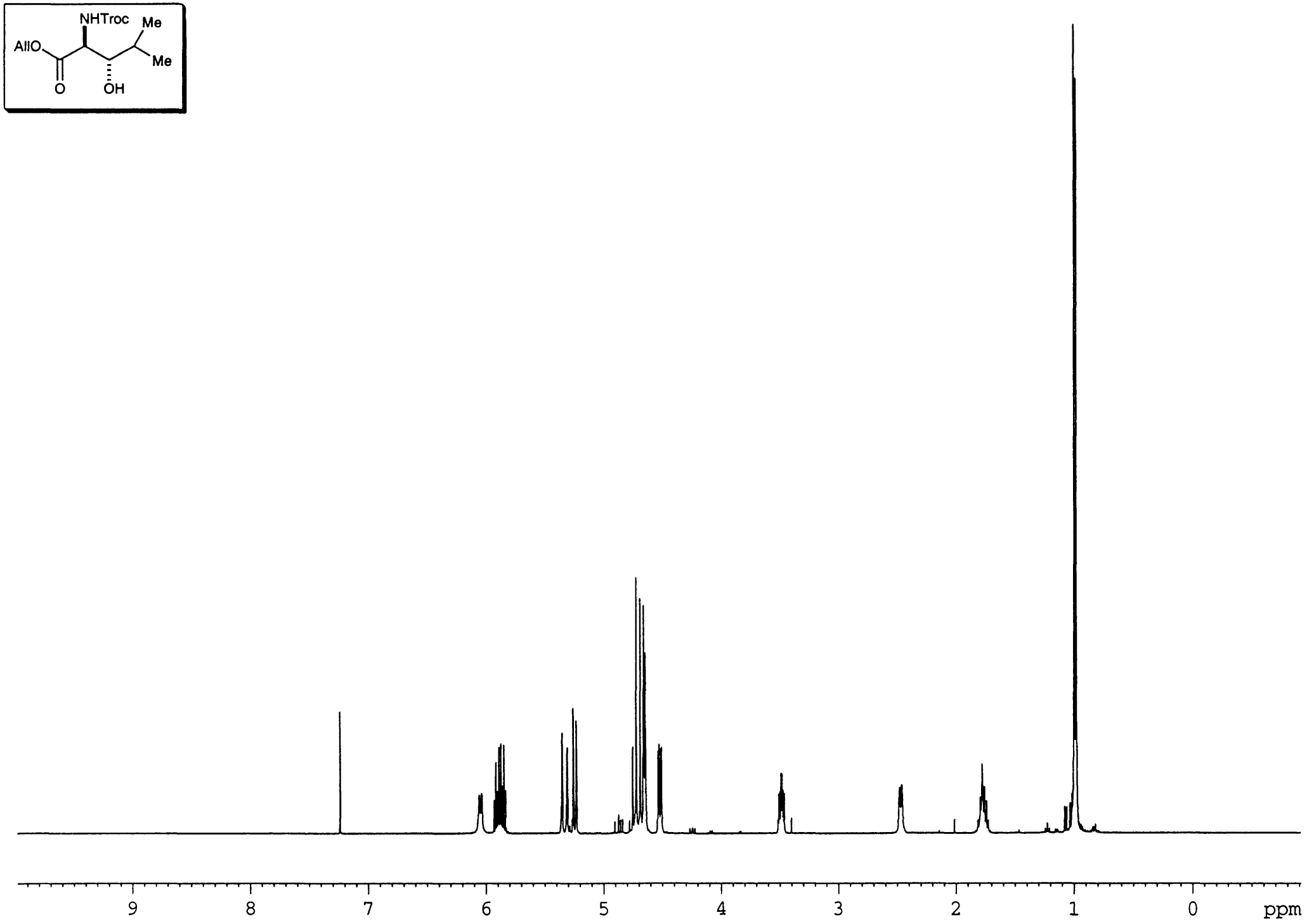

HO
O
N
Me
O
NHBoc

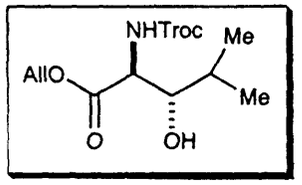
11
10
9
8
7
6
5
4
3
2
1
0
ppm


HO
O
N
Me
O
NHBoc

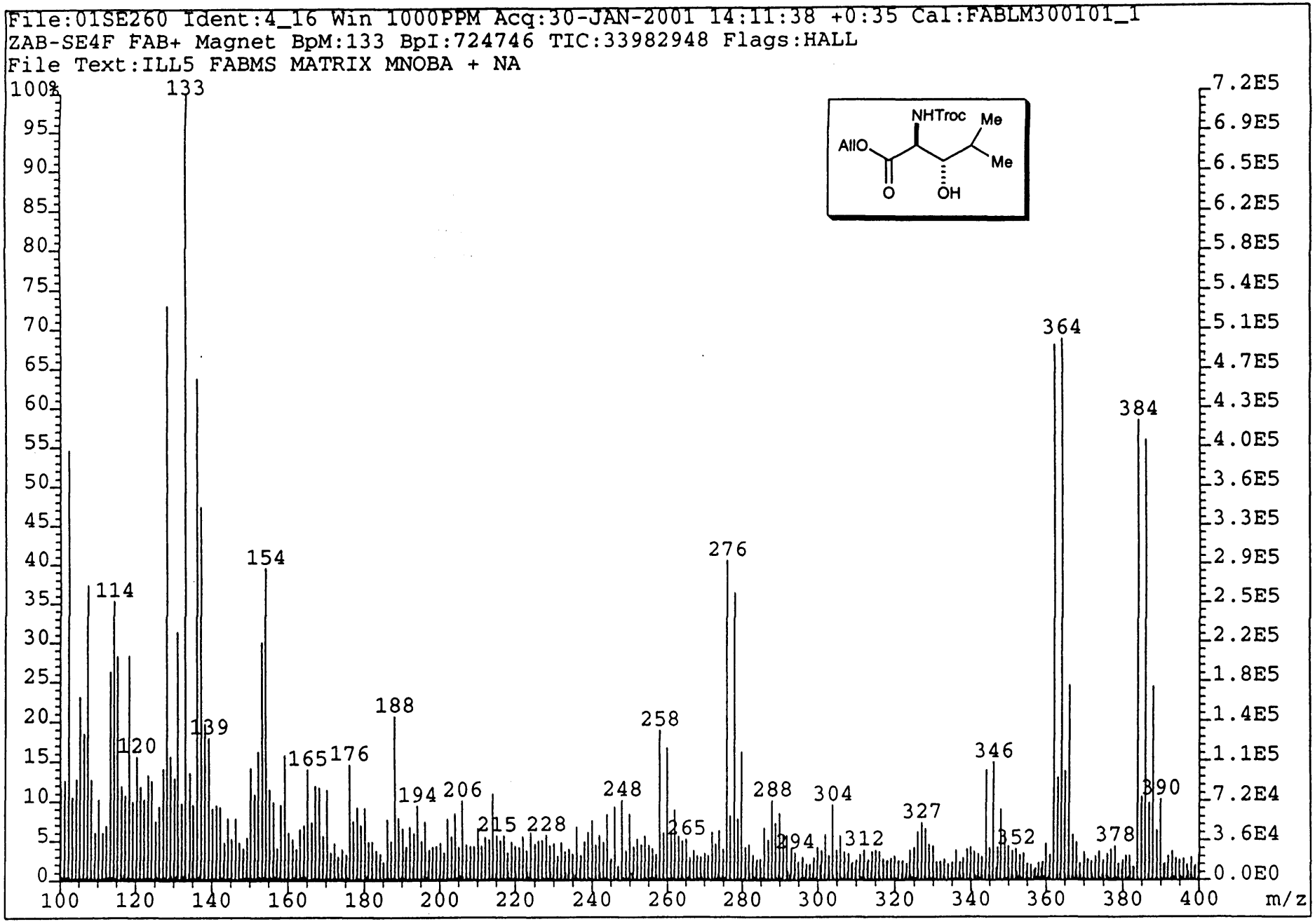
220
200
180
160
140
120
100
80
60
40
20
0
ppm

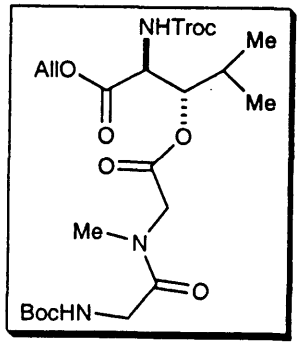

File:01SE2014 Ident:10_12 Win 1000PPM Acq:24-MAY-2001 11:38:10 +0:36 Cal:FABLM210501_1
ZAB-SE4F FAB+ Magnet BpM:147 BpI:9475414 TIC:68854056 Flags:HALL
File Text:I-LL-6 FABMS MATRIX MNOBA + NA
HO
O
N
Me
O
NHBoc
147
191
269
247
169
219
173
118
137
154
127
131
159
181
197
202
226
253
259
275
281
m/z


NHTroc
Me
AllO
Me
O
OH
9
8
7
6
5
4
3
2
1
0
ppm

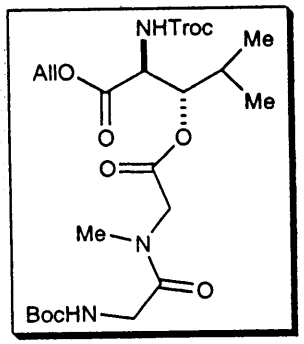

NHTroc
Me
AllO
Me
O
OH

220
200
180
160
140
120
100
80
60
40
20
0
ppm

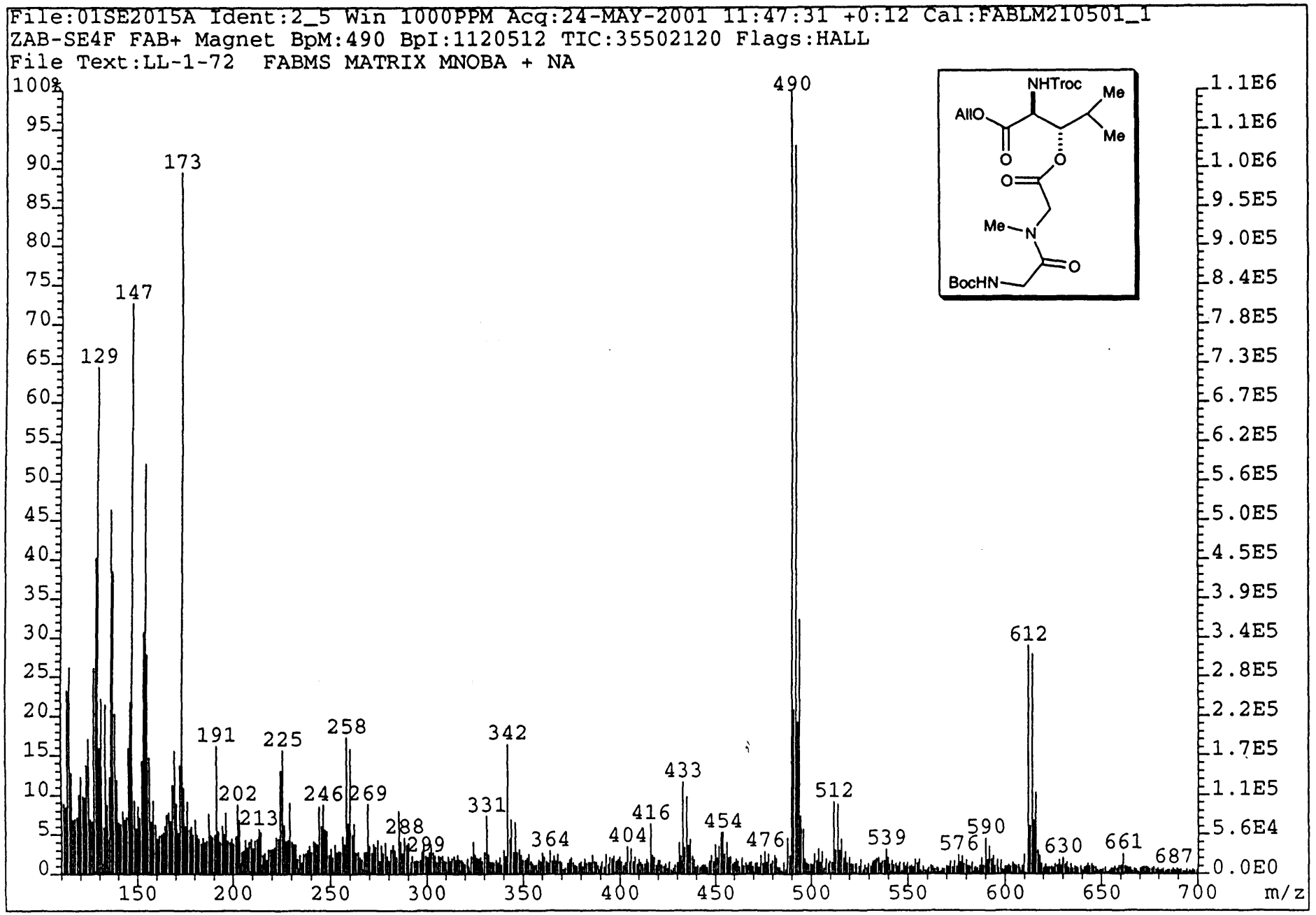

File:01SE260 Ident:4_16 Win 1000PPM Acq:30-JAN-2001 14:11:38 +0:35 Cal:FABLM300101_1
ZAB-SE4F FAB+ Magnet BpM:133 BpI:724746 TIC:33982948 Flags:HALL
File Text:ILL5 FABMS MATRIX MNOBA + NA
NHTroc
Me
AllO
Me
O
OH
133
154
114
139
120
165
176
188
194
206
215
228
248
258
265
276
288
294
304
312
327
346
352
364
378
384
390
m/z

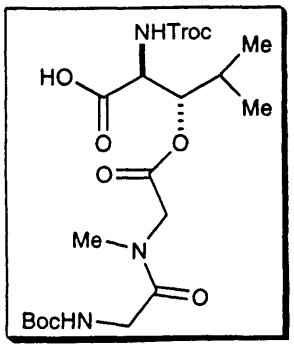

NHTroc
Me
Me
AllO
O
O
O
Me
N
O
BocHN

9
8
7
6
5
4
3
2
1
0
ppm

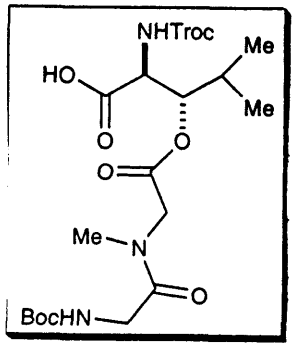

NHTroc
Me
Me
AllO
O
O
O
Me
N
O
BocHN

220
200
180
160
140
120
100
80
60
40
20
0
ppm

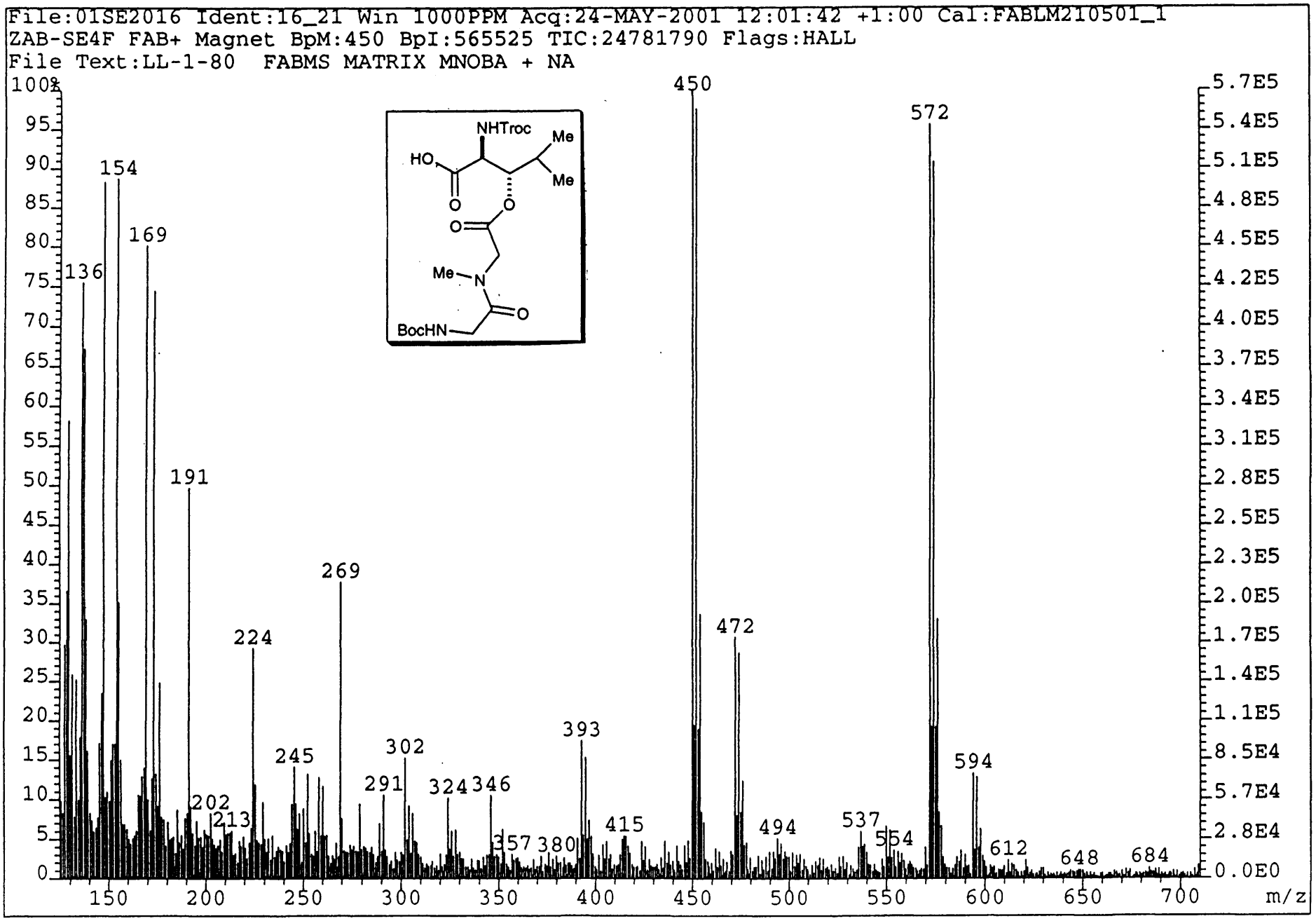

File:01SE2015A Ident:2_5 Win 1000PPM Acq:24-MAY-2001 11:47:31 +0:12 Cal:FABLM210501_1
ZAB-SE4F FAB+ Magnet BpM:490 BpI:1120512 TIC:35502120 Flags:HALL
File Text:LL-1-72 FABMS MATRIX MNOBA + NA
NHTroc
Me
Me
AllO
O
O
O
Me
N
O
BocHN
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
1.1E6
1.1E6
1.0E6
9.5E5
9.0E5
8.4E5
7.8E5
7.3E5
6.7E5
6.2E5
5.6E5
5.0E5
4.5E5
3.9E5
3.4E5
2.8E5
2.2E5
1.7E5
1.1E5
5.6E4
0.0E0
490
173
147
129
612
191
225
258
342
433
512
202
213
246
269
288
299
331
364
404
416
454
476
539
576
590
630
661
687
150
200
250
300
350
400
450
500
550
600
650
700
m/z

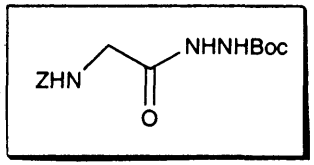

NHTroc
Me
Me
HO
O
O
O
Me
N
O
BocHN

11
10
9
8
7
6
5
4
3
2
1
0
ppm

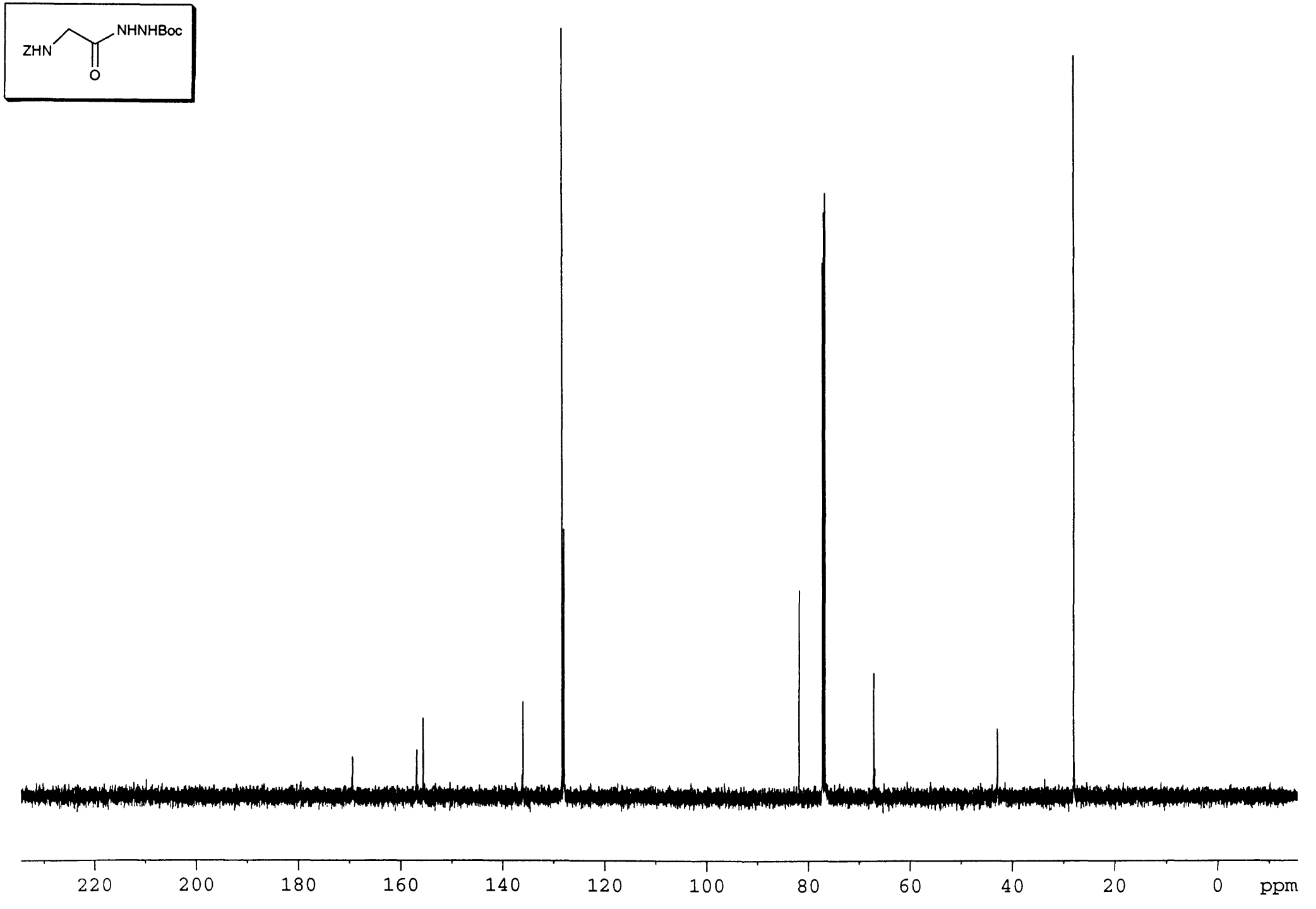

NHTroc
Me
HO
Me
O
O
O
Me
N
O
BocHN

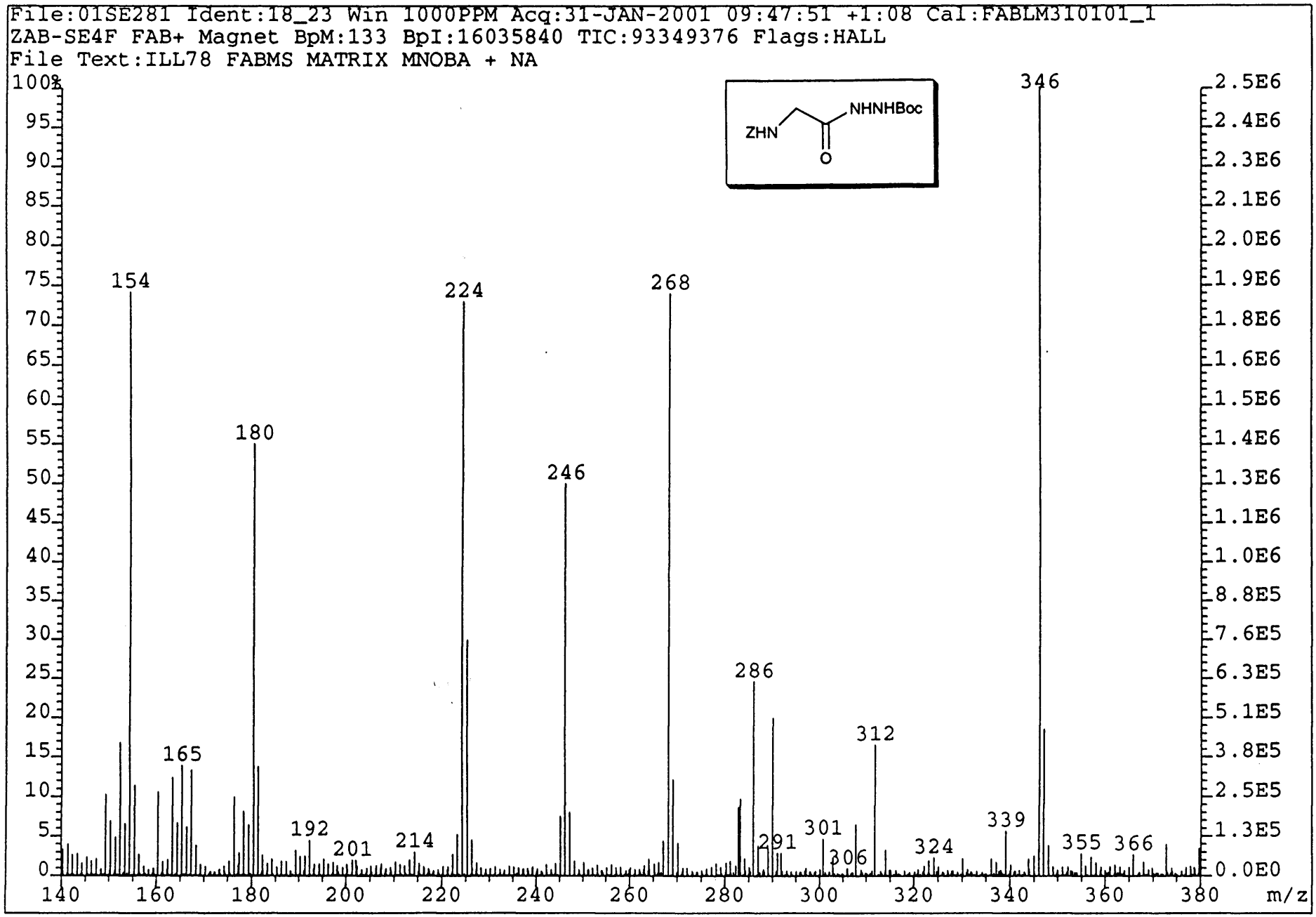
220
200
180
160
140
120
100
80
60
40
20
0
ppm

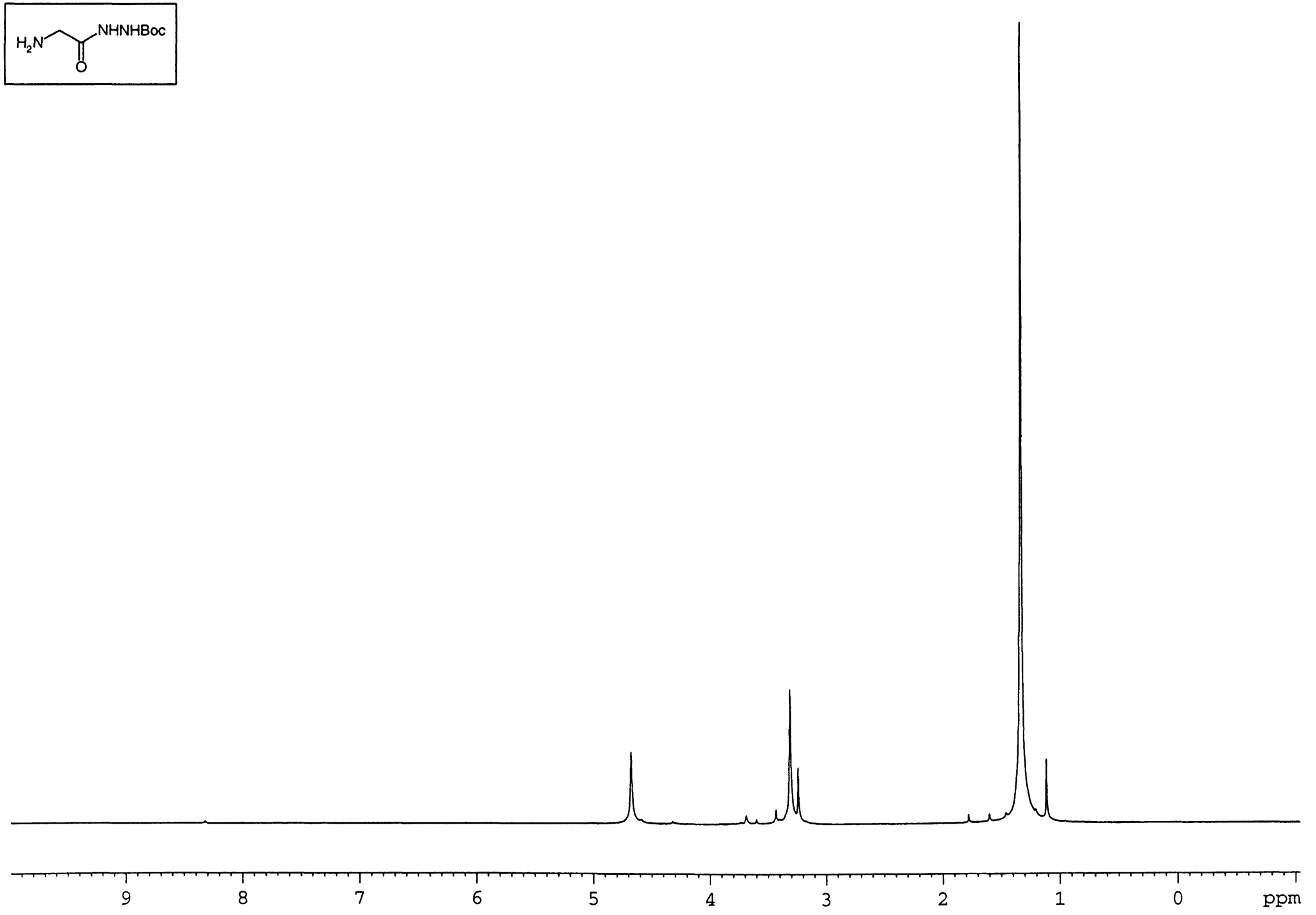

File:01SE2016 Ident:16_21 Win 1000PPM Acq:24-MAY-2001 12:01:42 +1:00 Cal:FABLM210501_1
ZAB-SE4F FAB+ Magnet BpM:450 BpI:565525 TIC:24781790 Flags:HALL
File Text:LL-1-80 FABMS MATRIX MNOBA + NA
NHTroc
Me
Me
HO
O
O
O
O
Me
N
O
BocHN
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
5.7E5
5.4E5
5.1E5
4.8E5
4.5E5
4.2E5
4.0E5
3.7E5
3.4E5
3.1E5
2.8E5
2.5E5
2.3E5
2.0E5
1.7E5
1.4E5
1.1E5
8.5E4
5.7E4
2.8E4
0.0E0
150
200
250
300
350
400
450
500
550
600
650
700
m/z
450
572
154
169
136
191
269
224
472
393
245
302
594
291
324
346
202
213
415
494
537
554
612
648
684
357
380

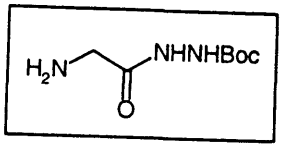

NHNHBoc
ZHN
O

9
8
7
6
5
4
3
2
1
0
ppm

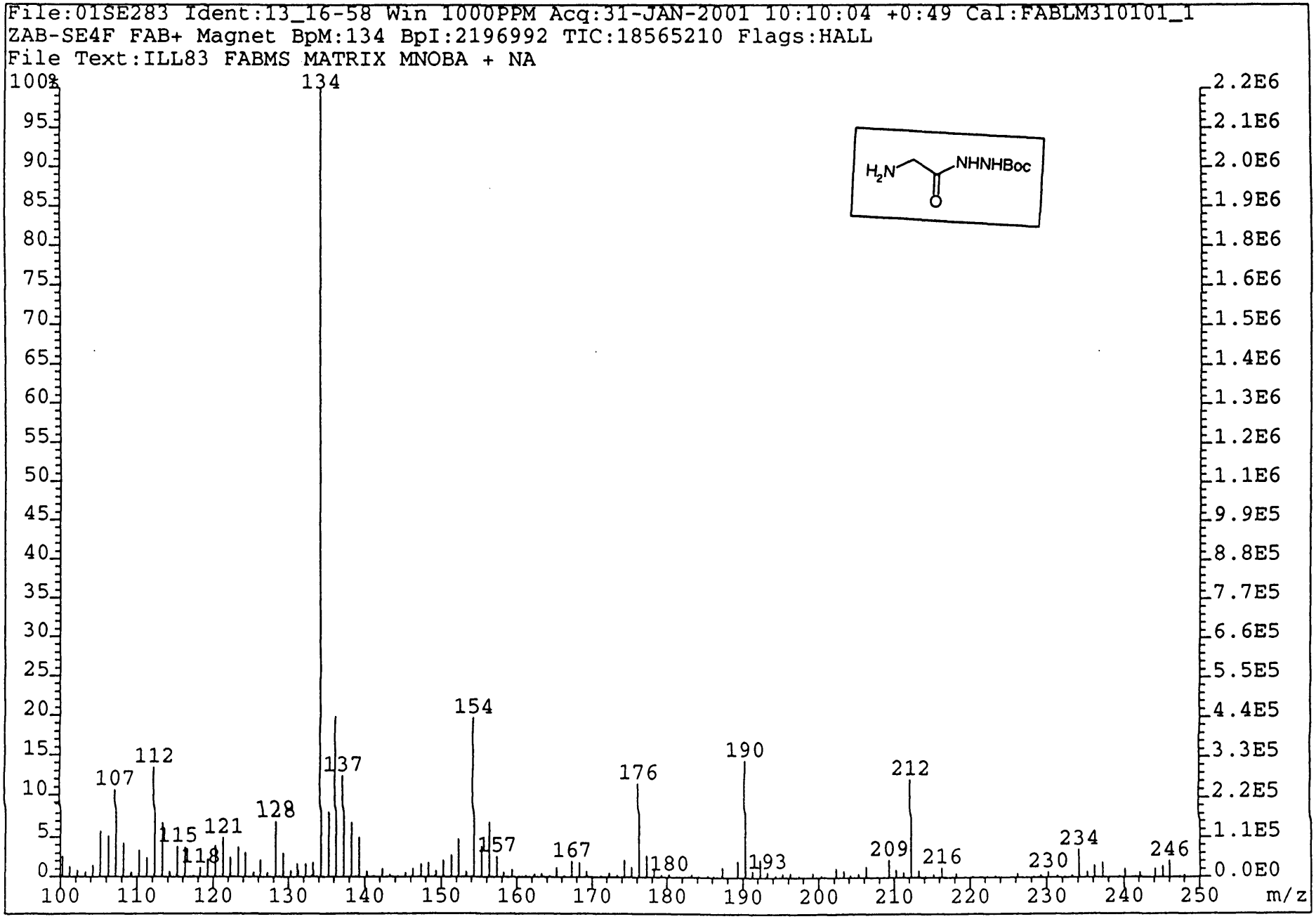

ZHN
NHNHBoc
O
220
200
180
160
140
120
100
80
60
40
20
0
ppm


File:01SE281 Ident:18_23 Win 1000PPM Acq:31-JAN-2001 09:47:51 +1:08 Cal:FABLM310101_1
ZAB-SE4F FAB+ Magnet BpM:133 BpI:16035840 TIC:93349376 Flags:HALL
File Text:ILL78 FABMS MATRIX MNOBA + NA
ZHN
NHNHBoc
O
346
154
224
268
180
246
286
312
165
192
201
214
291
301
306
324
339
355
366
m/z

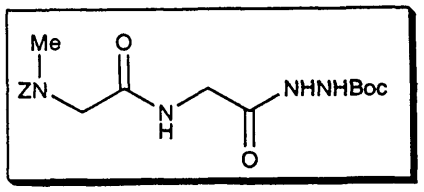

H2N
NHNHBoc
O
9
8
7
6
5
4
3
2
1
0
ppm


H2N
NHNHBoc
O

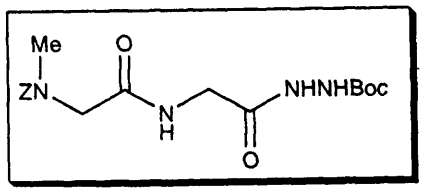
220
200
180
160
140
120
100
80
60
40
20
0
pp


File:01SE283 Ident:13_16-58 Win 1000PPM Acq:31-JAN-2001 10:10:04 +0:49 Cal:FABLM310101_1
ZAB-SE4F FAB+ Magnet BpM:134 BpI:2196992 TIC:18565210 Flags:HALL
File Text:ILL83 FABMS MATRIX MNOBA + NA
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
2.2E6
2.1E6
2.0E6
1.9E6
1.8E6
1.6E6
1.5E6
1.4E6
1.3E6
1.2E6
1.1E6
9.9E5
8.8E5
7.7E5
6.6E5
5.5E5
4.4E5
3.3E5
2.2E5
1.1E5
0.0E0
134
154
190
212
112
137
176
107
128
121
115
118
157
167
180
193
209
216
230
234
246
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
m/z
H2N
NHNHBoc
O

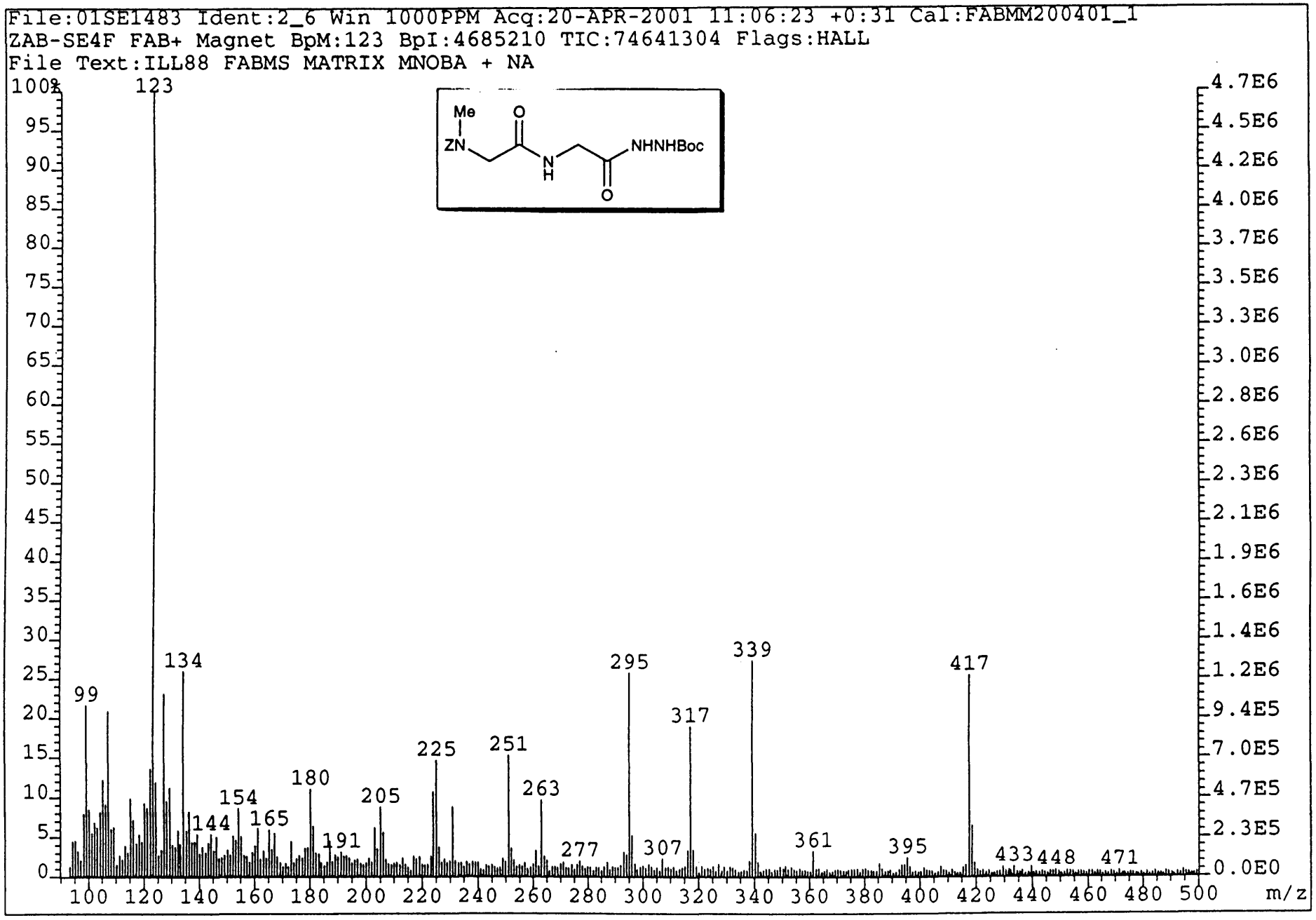
1LL88

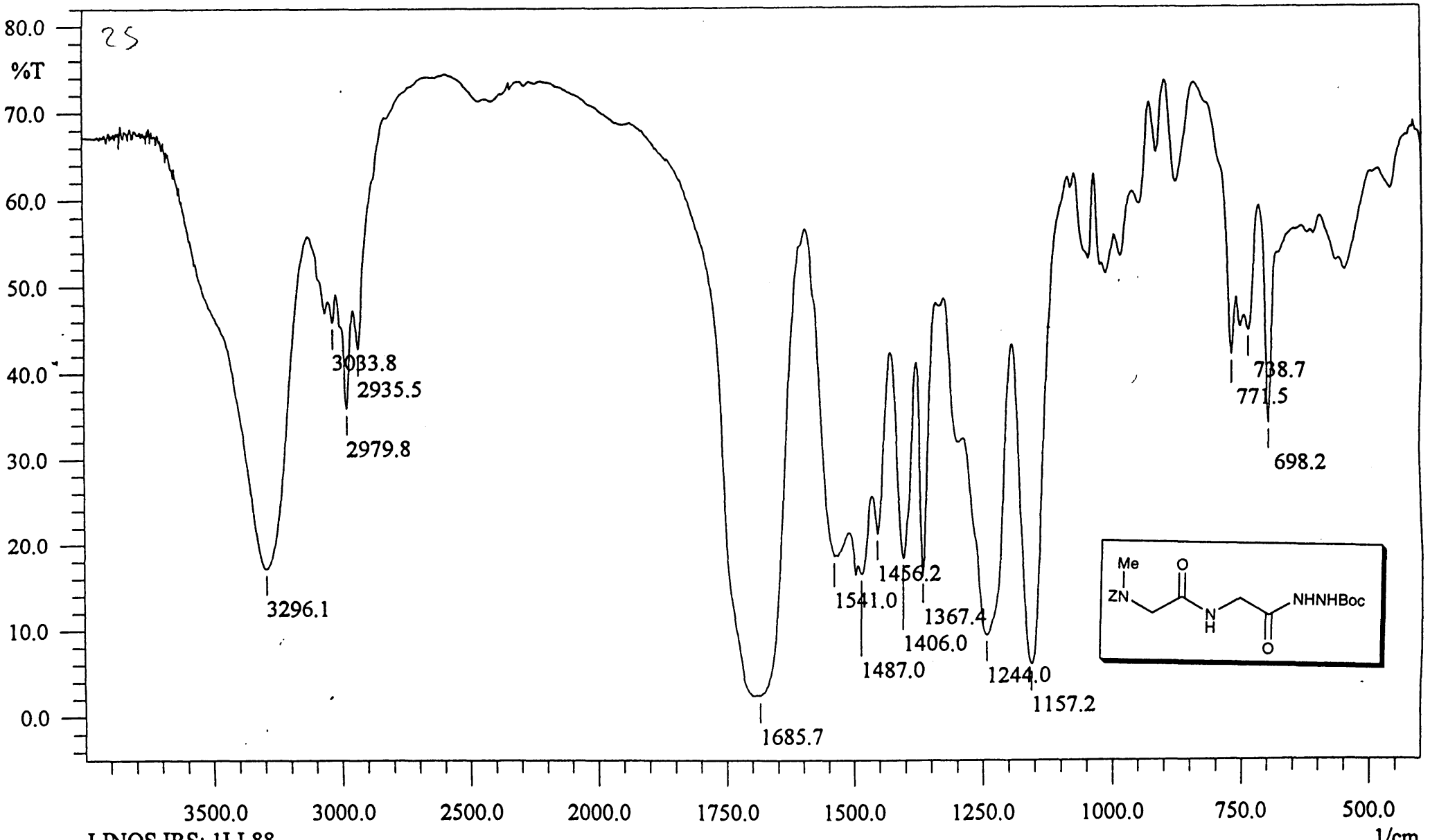


1LL88C


Me
ZN
O
N
H
O
NHNHBoc

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

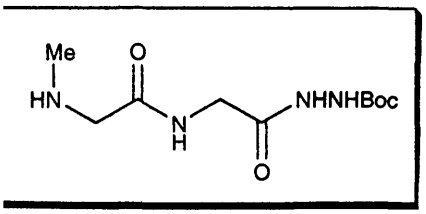

File:01SE1483 Ident:2_6 Win 1000PPM Acq:20-APR-2001 11:06:23 +0:31 Cal:FABMM200401_1
ZAB-SE4F FAB+ Magnet BpM:123 BpI:4685210 TIC:74641304 Flags:HALL
File Text:ILL88 FABMS MATRIX MNOBA + NA
Me
O
ZN
N
H
NHNHBoc
O
123
134
99
144
154
165
180
191
205
225
251
263
277
295
307
317
339
361
395
417
433
448
471
m/z



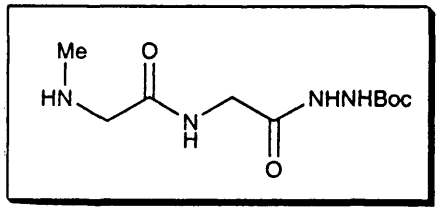
LINOS.IRS: 1LL88

Date:	01/02/01	Time:	12:55:30	NScans:	20	
Type:	HYPER IR	User:	A20923500085 Shimadz		Detector:	standard
Abscissa:	1/cm	Ordinate:	%T	Apodization:	Happ	
Min:	401.17	Max:	3998.16	Range:	1/cm	
Ndp:	1866	Data Interval:	1.92868	Resolution:	4.0	
Gain:	auto	Aperture:	auto	Mirror Speed:	2.8(low)	

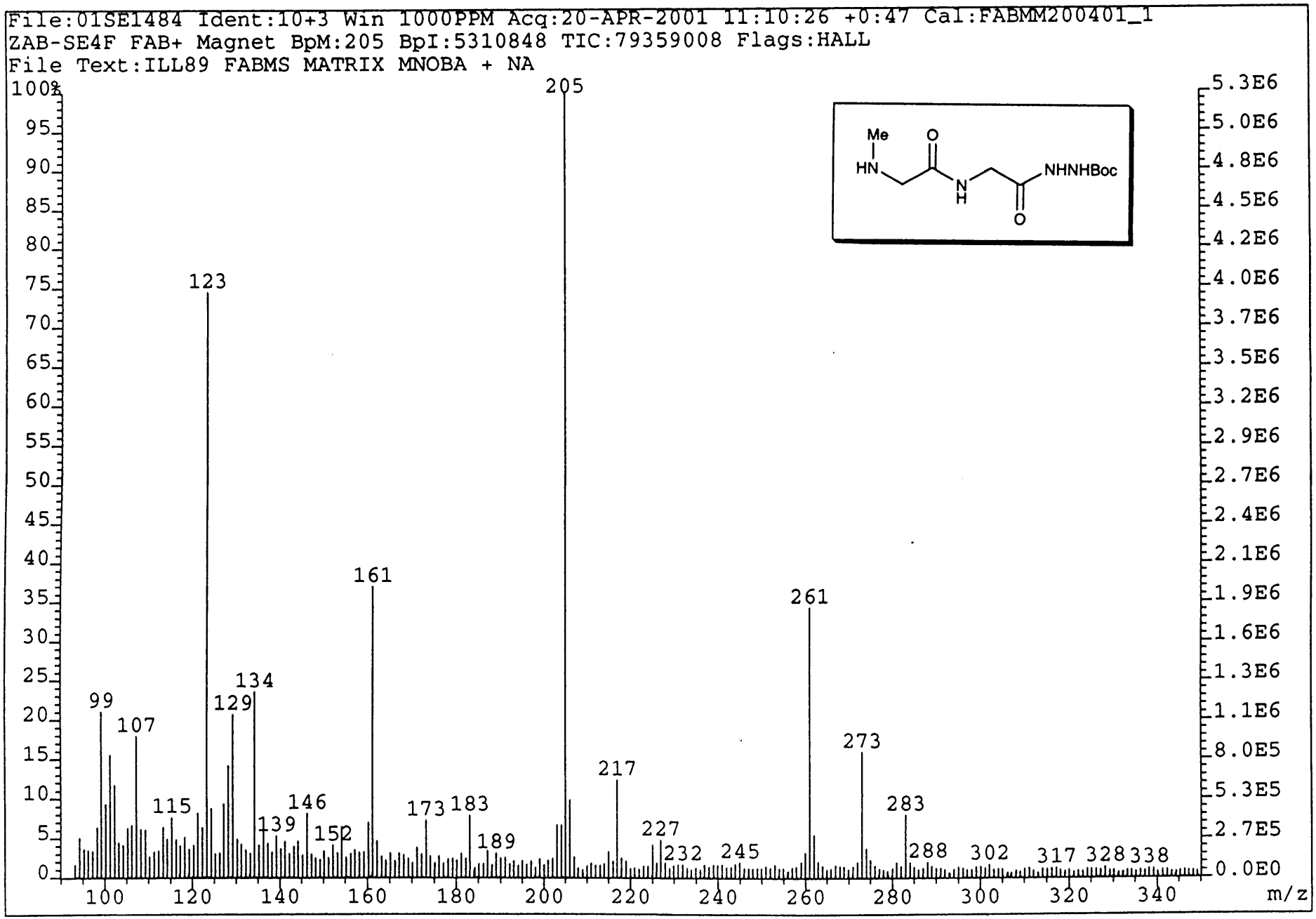
1LL89

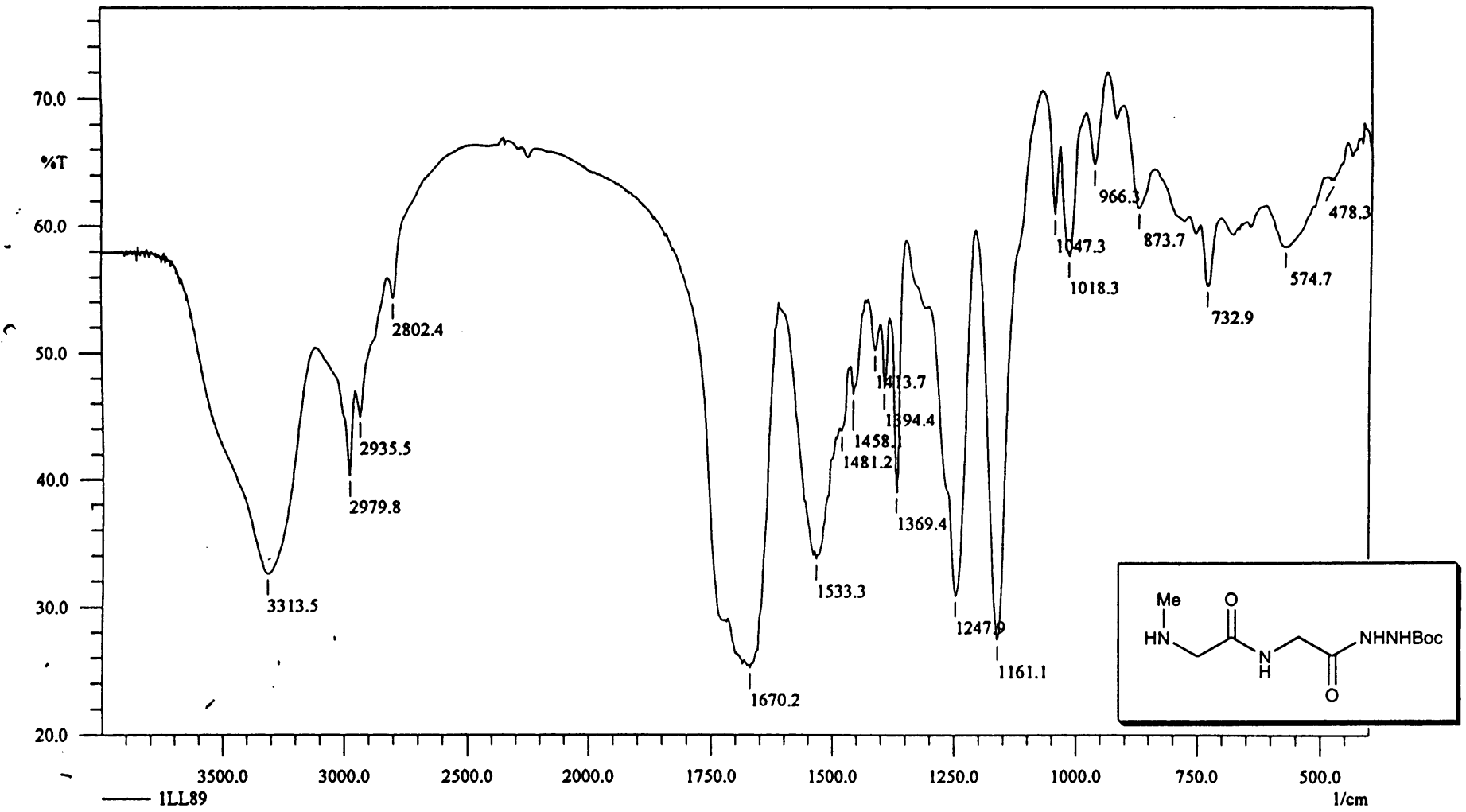


1LL89C

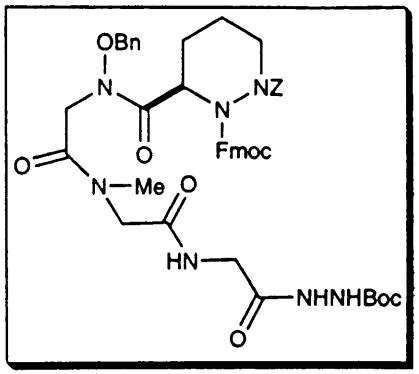

Me
O
HN
N
H
NHNHBoc
O

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

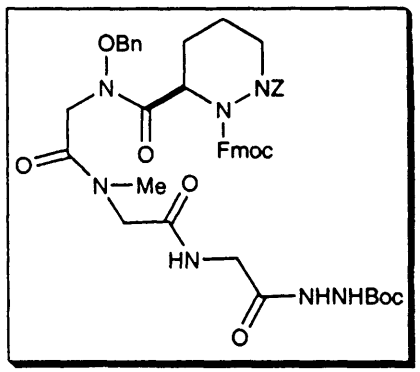

File:01SE1484 Ident:10+3 Win 1000PPM Acq:20-APR-2001 11:10:26 +0:47 Cal:FABMM200401_1
ZAB-SE4F FAB+ Magnet BpM:205 BpI:5310848 TIC:79359008 Flags:HALL
File Text:ILL89 FABMS MATRIX MNOBA + NA
Me
HN
O
N
H
O
NHNHBoc
205
123
161
261
273
99
107
115
129
134
139
146
152
173
183
189
217
227
232
245
283
288
302
317
328
338
m/z


70.0
%T
60.0
50.0
40.0
30.0
20.0
3313.5
2979.8
2935.5
2802.4
1670.2
1533.3
1481.2
1458.1
1413.7
1394.4
1369.4
1247.9
1161.1
1047.3
1018.3
966.3
873.7
732.9
574.7
478.3
Me
HN
O
N
H
NHNHBoc
O
3500.0
3000.0
2500.0
2000.0
1750.0
1500.0
1250.0
1000.0
750.0
500.0
1/cm
1LL89

1-LL-66

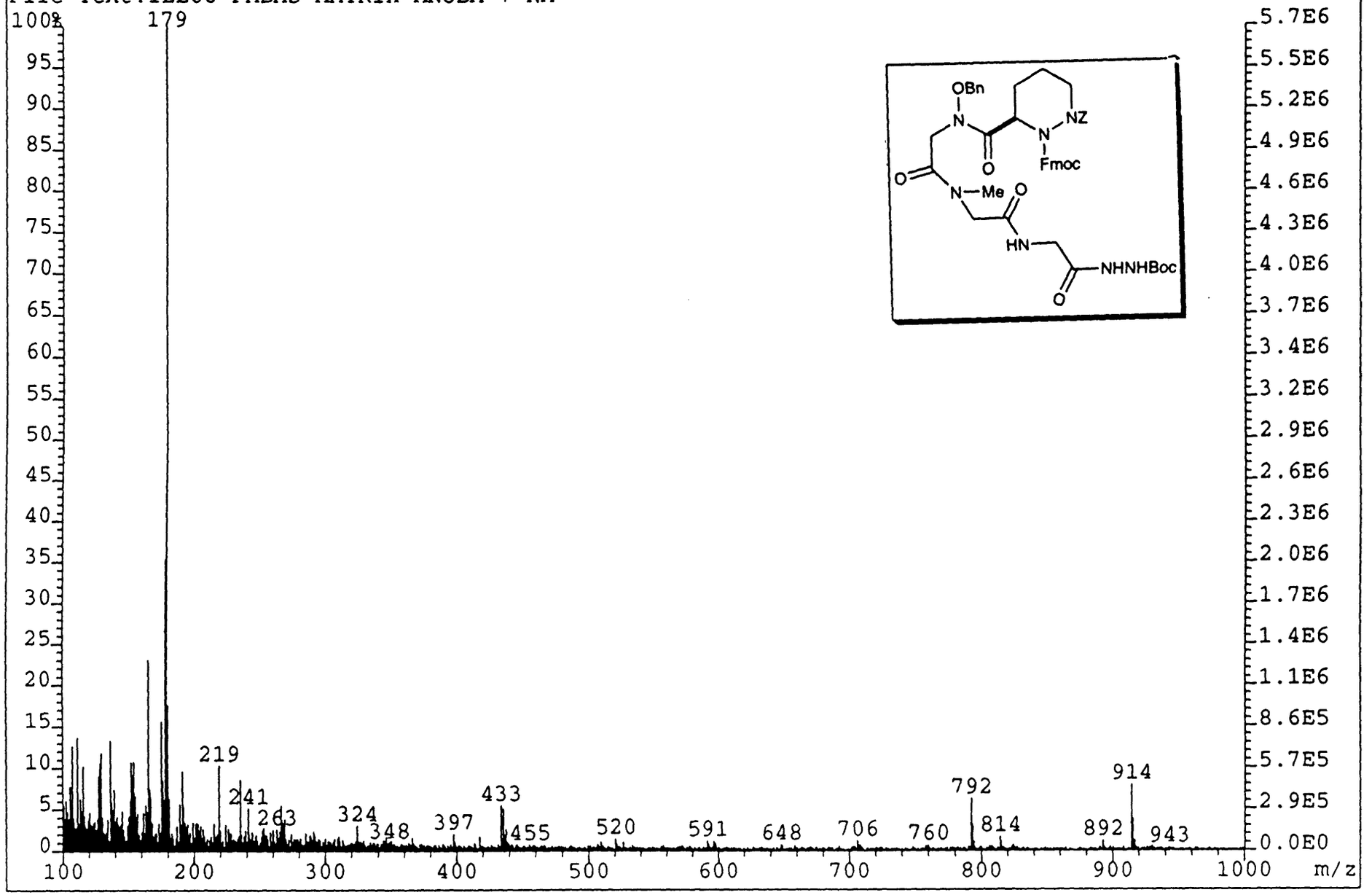


1-LL-66C


OBn
N
NZ
N
Fmoc
O
O
N—Me
O
HN
NHNHBoc
O

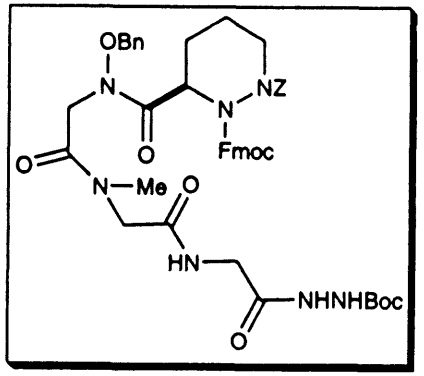
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

File:01SE1478 Ident:5_7 Win 1000PPM Acq:20-APR-2001 10:36:34 +0:44 Cal:FABMM200401_1
ZAB-SE4F FAB+ Magnet BpM:179 BpI:5741910 TIC:81979488 Flags:HALL
File Text:ILL66 FABMS MATRIX MNOBA + NA



PERKIN ELMER

32



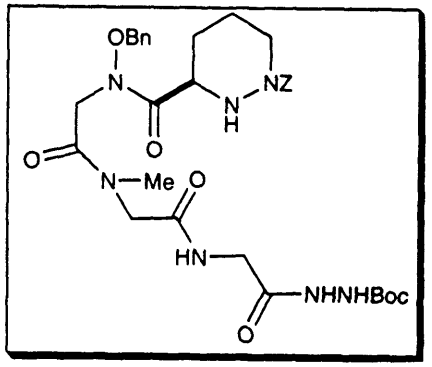
84.37
%T

-14.57

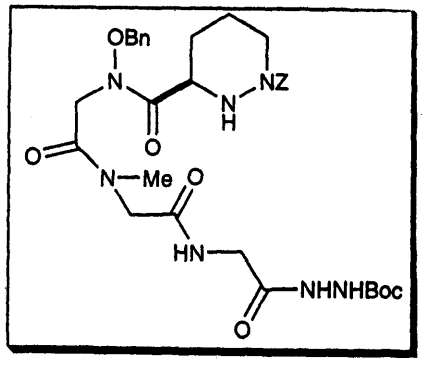
3500 3000 2500 2000 1500 1000 cm^{-1} 500

01/02/19 19:04
X: 16 scans, 4.0cm-1

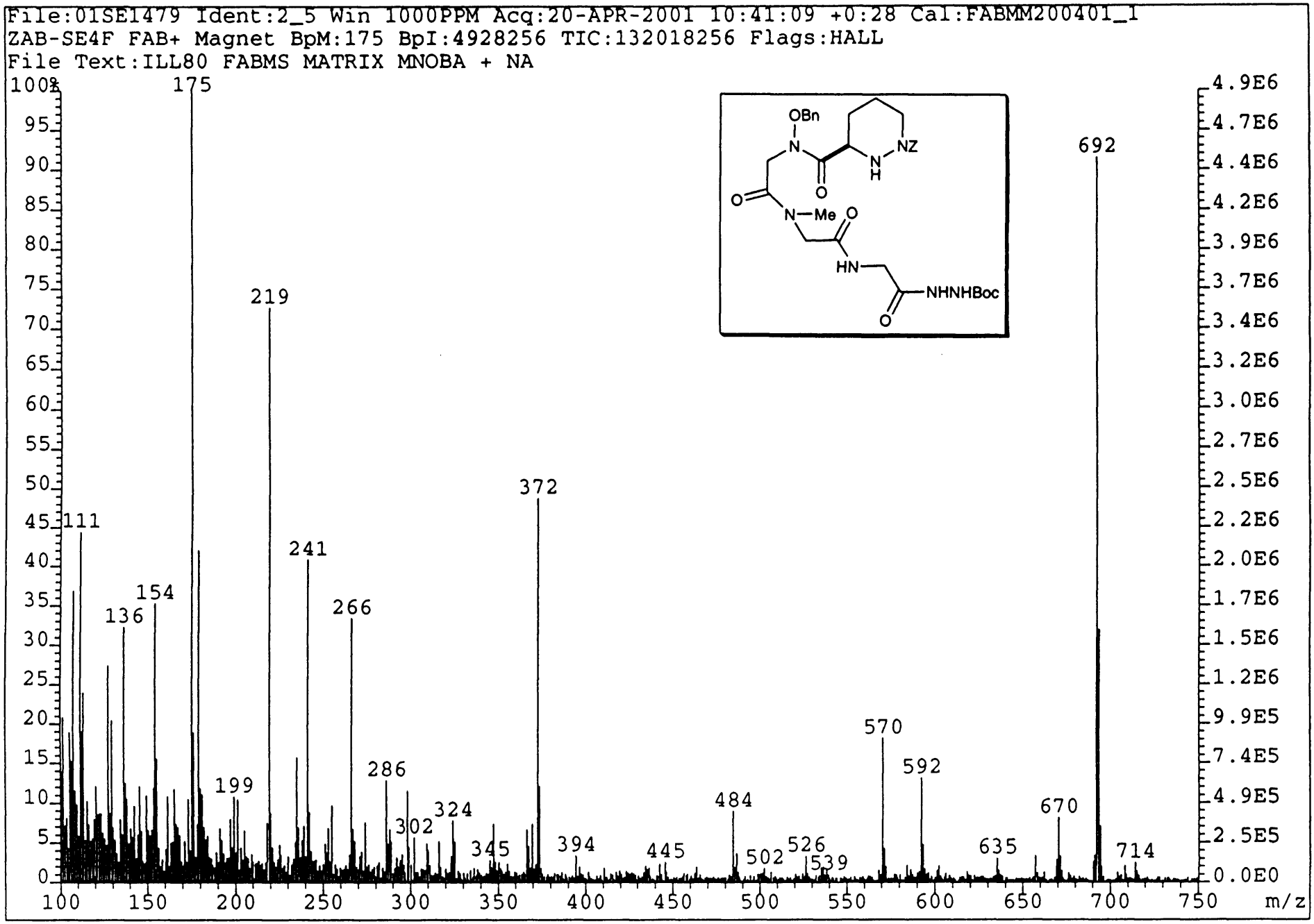
1-LL-80



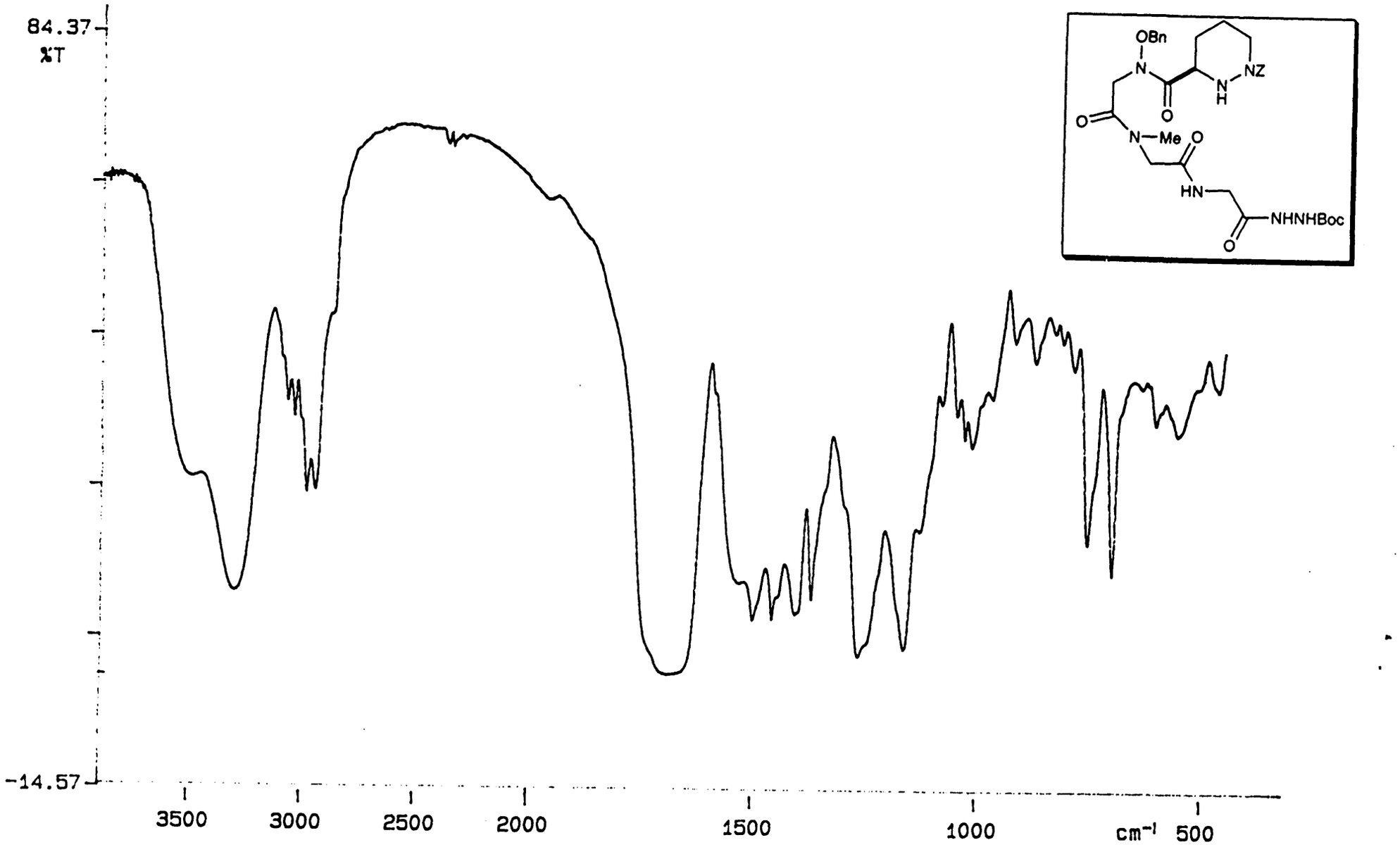
1-LL-80C


OBn
N
NZ
N
H
O
O
N—Me
O
HN
NHNHBoc
O

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


File:01SE1479 Ident:2_5 Win 1000PPM Acq:20-APR-2001 10:41:09 +0:28 Cal:FABMM200401_1
ZAB-SE4F FAB+ Magnet BpM:175 BpI:4928256 TIC:132018256 Flags:HALL
File Text:ILL80 FABMS MATRIX MNOBA + NA
OBn
N
NZ
N
H
O
O
N—Me
O
HN
NHNHBoc
O
175
692
219
372
111
241
154
266
136
286
199
324
302
345
394
445
484
502
526
539
570
592
635
670
714
m/z

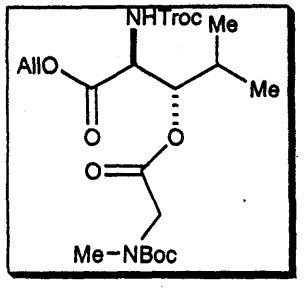
PERKIN ELMER



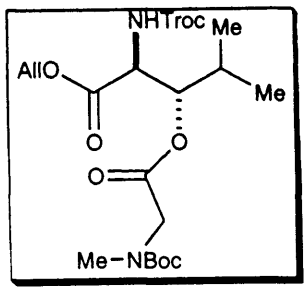
01/02/20 12:22
X: 16 scans, 4.0cm-1

3 4 4 30

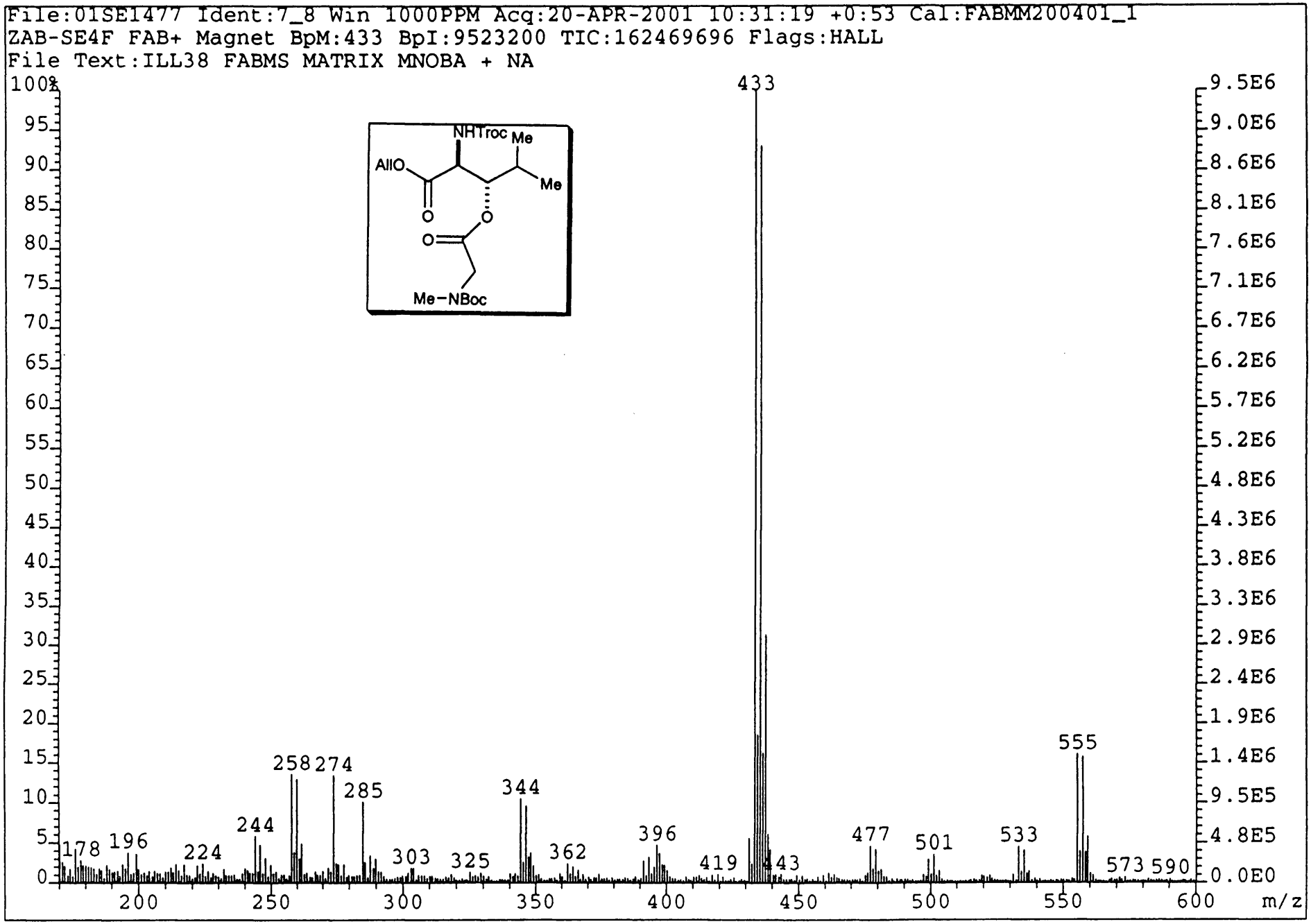
ILL38

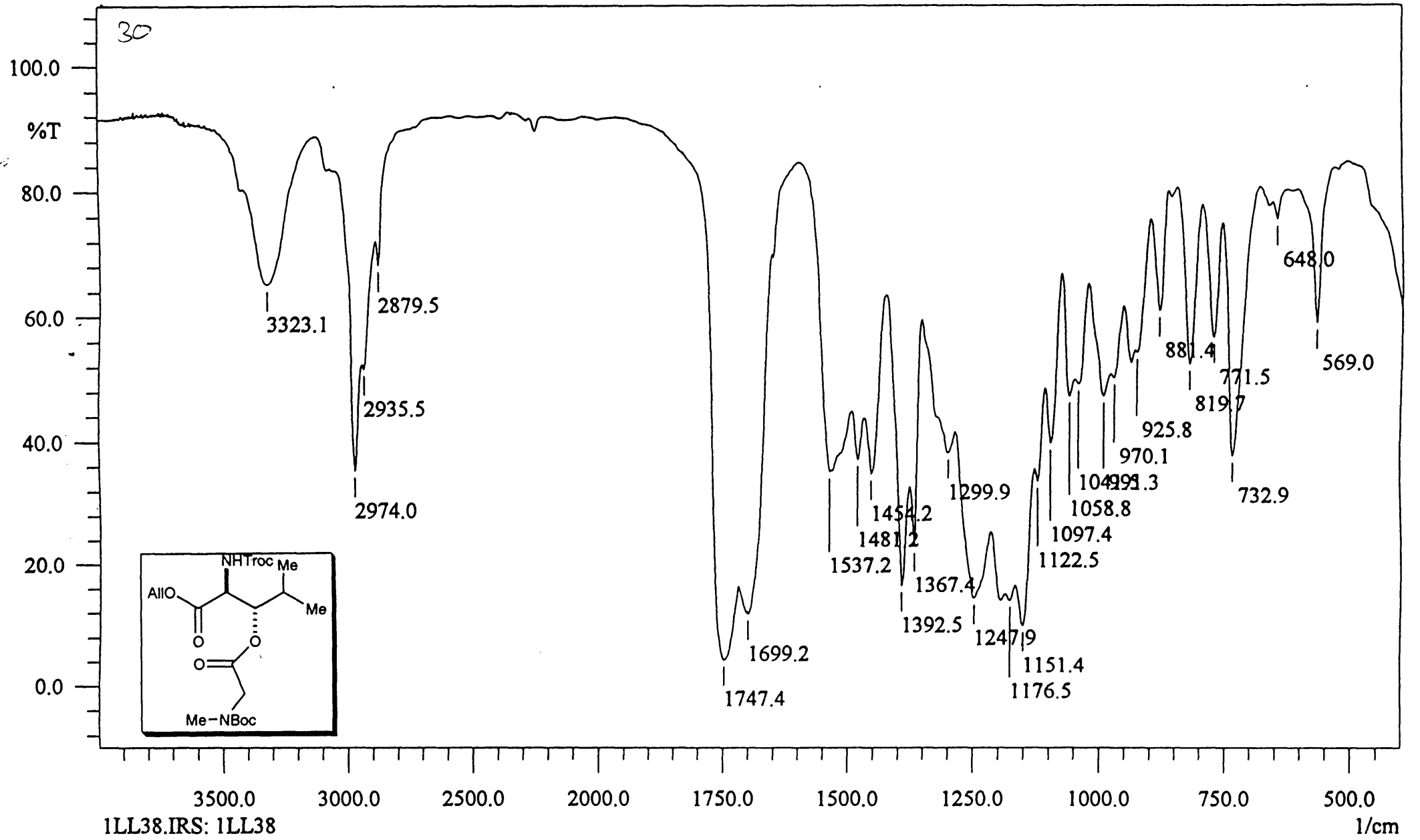


ILL38C


NHTroc
Me
AllO
Me
O
O
O
Me–NBoc

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

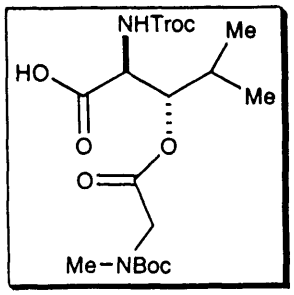

File:01SE1477 Ident:7_8 Win 1000PPM Acq:20-APR-2001 10:31:19 +0:53 Cal:FABMM200401_1
ZAB-SE4F FAB+ Magnet BpM:433 BpI:9523200 TIC:162469696 Flags:HALL
File Text:ILL38 FABMS MATRIX MNOBA + NA
NHTroc
Me
AllO
Me
O
O
O
Me-NBoc
433
555
258
274
285
344
244
396
178
196
224
303
325
362
419
443
477
501
533
573
590
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
9.5E6
9.0E6
8.6E6
8.1E6
7.6E6
7.1E6
6.7E6
6.2E6
5.7E6
5.2E6
4.8E6
4.3E6
3.8E6
3.3E6
2.9E6
2.4E6
1.9E6
1.4E6
9.5E5
4.8E5
0.0E0
200
250
300
350
400
450
500
550
600
m/z



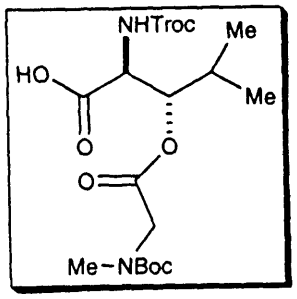
1LL38.IRS: 1LL38

Date:	02/02/01	Time:	17:58:14	NScans:	20	
Type:	HYPER IR	User:	A20923500085 Shimadz	Detector:	standard	
Abscissa:	1/cm	Ordinate:	%T	Apodization:	Happ	
Min:	401.17	Max:	3998.16	Range:	1/cm	
Ndp:	1866	Data Interval:	1.92868	Resolution:	4.0	
Gain:	auto	Aperture:	auto	Mirror Speed:	2.8(low)	

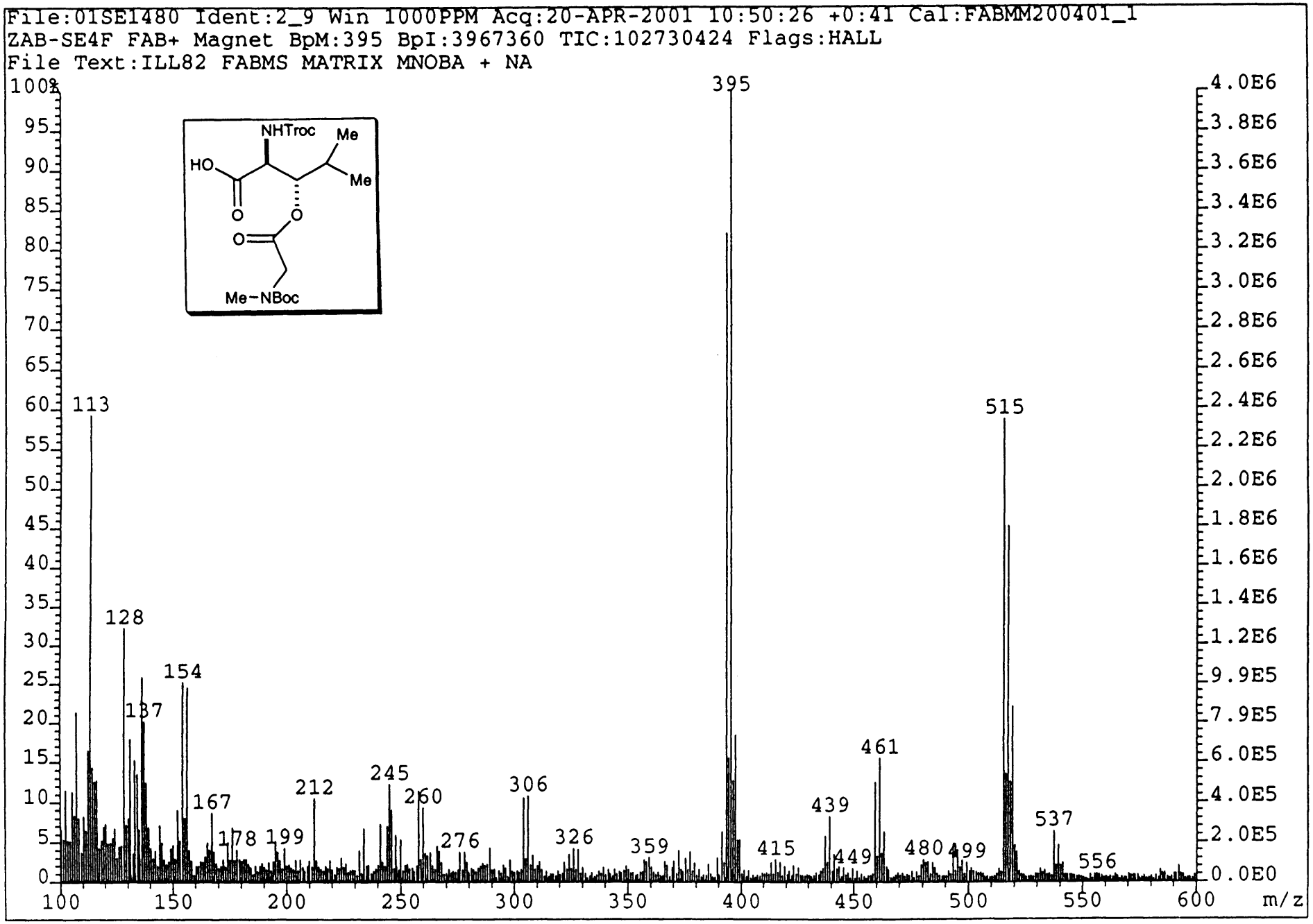
1-LL-82

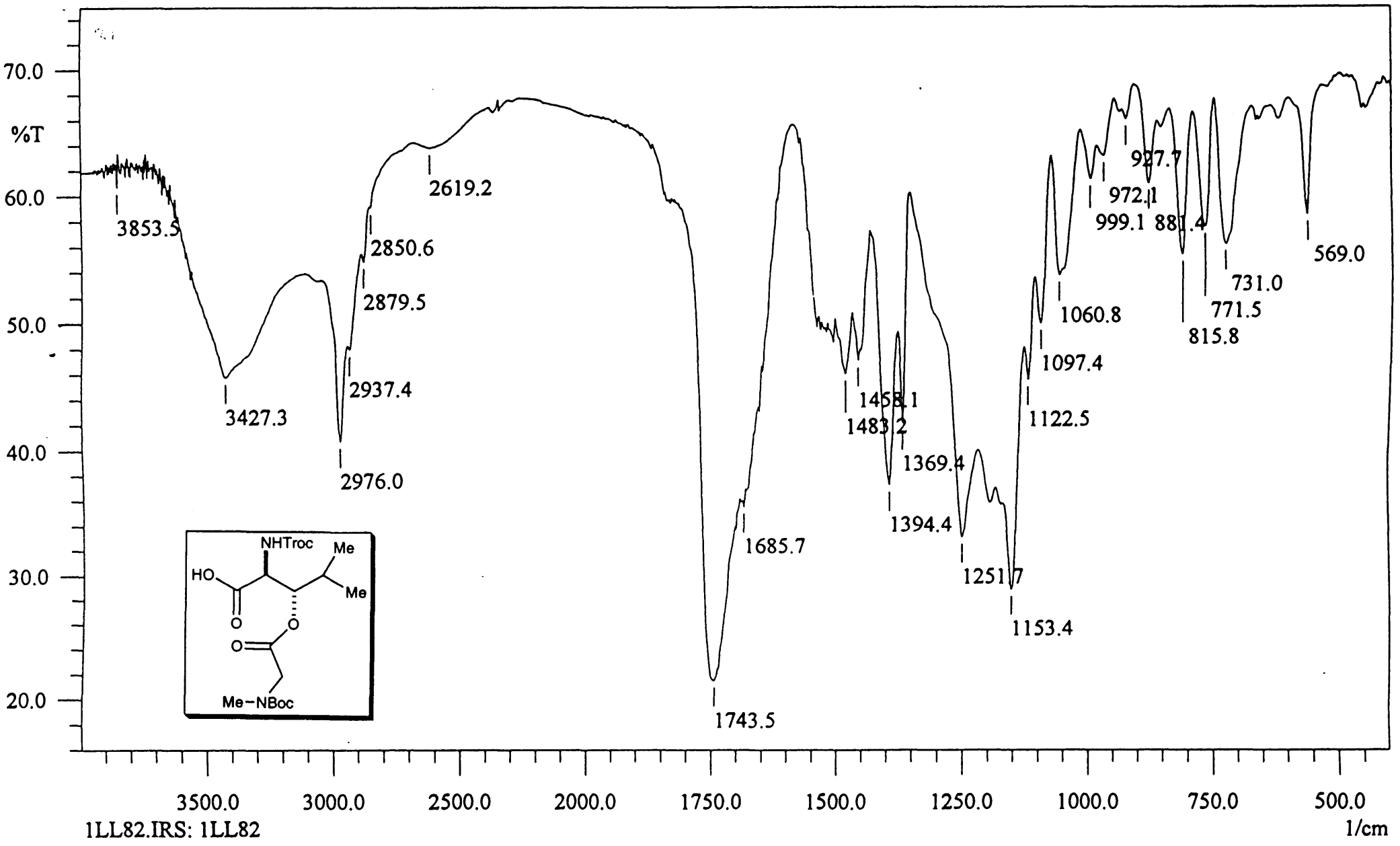


ILL82C


NHTroc
Me
HO
Me
O
O
O
Me-NBoc

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

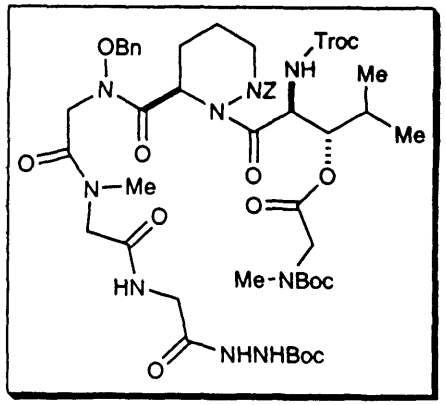

File:01SE1480 Ident:2_9 Win 1000PPM Acq:20-APR-2001 10:50:26 +0:41 Cal:FABMM200401_1
ZAB-SE4F FAB+ Magnet BpM:395 BpI:3967360 TIC:102730424 Flags:HALL
File Text:ILL82 FABMS MATRIX MNOBA + NA
NHTroc
Me
Me
HO
O
O
O
Me-NBoc
395
113
515
128
154
137
167
212
245
260
306
439
461
537
178
199
276
326
359
415
449
480
499
556
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
4.0E6
3.8E6
3.6E6
3.4E6
3.2E6
3.0E6
2.8E6
2.6E6
2.4E6
2.2E6
2.0E6
1.8E6
1.6E6
1.4E6
1.2E6
9.9E5
7.9E5
6.0E5
4.0E5
2.0E5
0.0E0
100
150
200
250
300
350
400
450
500
550
600
m/z



1LL82.IRS: 1LL82

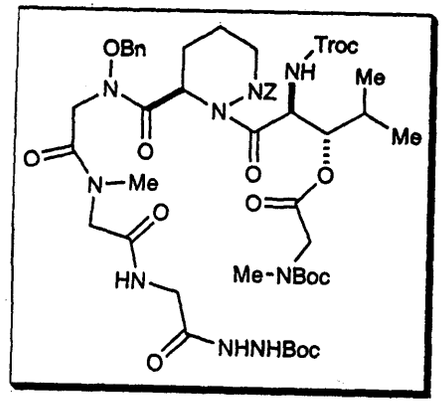
Date:	05/02/01	Time:	11:02:29	NScans:	20	
Type:	HYPER IR	User:	A20923500085 Shimadz	Detector:	standard	
Abscissa:	1/cm	Ordinate:	%T	Apodization:	Happ	
Min:	401.17	Max:	3998.16	Range:	1/cm	
Ndp:	1866	Data Interval:	1.92868	Resolution:	4.0	
Gain:	auto	Aperture:	auto	Mirror Speed:	2.8(low)	

1-LL-84

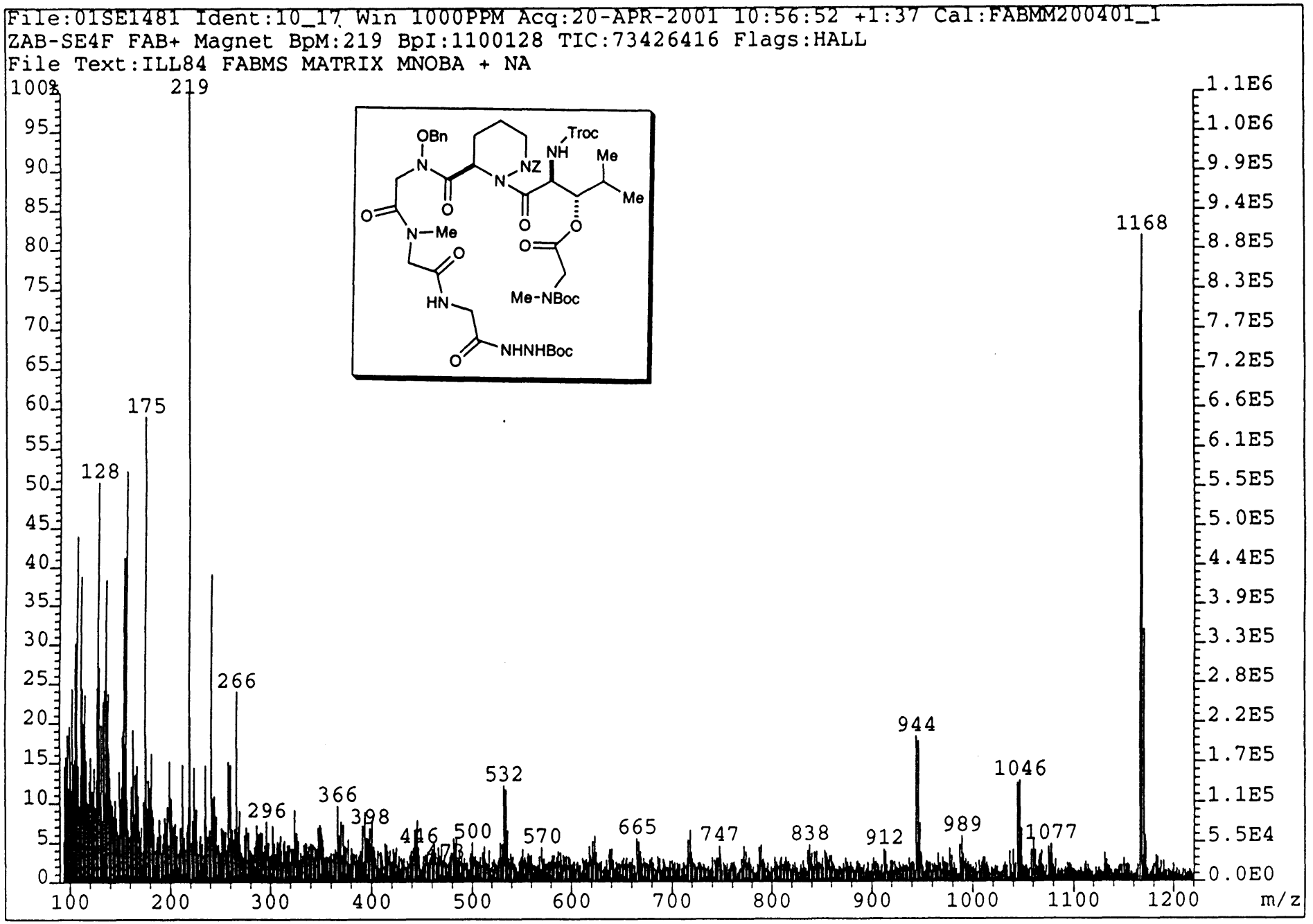

OBn
N
Troc
NH
Me
NZ
N
Me
O
O
O
O
N–Me
O
O
Me-NBoc
HN
NHNHBoc
O

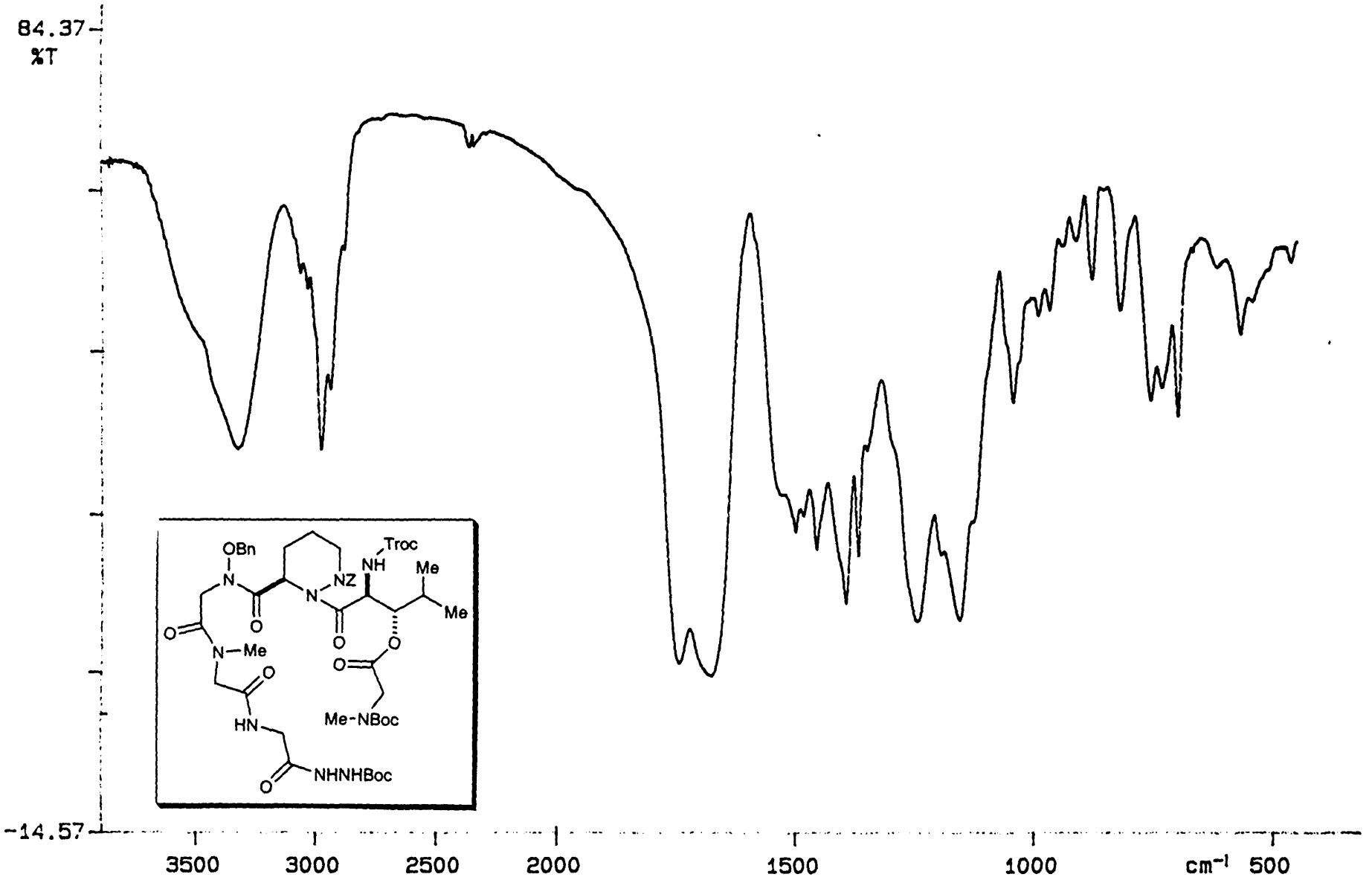
10
9
8
7
6
5
4
3
2
1
0
ppm

1-LL-84C


OBn
Troc
NH
Me
NZ
Me
N–Me
Me-NBoc
HN
NHNHBoc

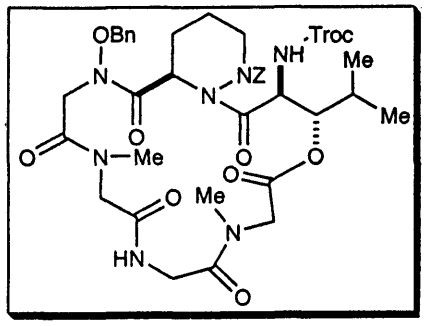
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


File:01SE1481 Ident:10_17 Win 1000PPM Acq:20-APR-2001 10:56:52 +1:37 Cal:FABMM200401_1
ZAB-SE4F FAB+ Magnet BpM:219 BpI:1100128 TIC:73426416 Flags:HALL
File Text:ILL84 FABMS MATRIX MNOBA + NA
OBn
Troc
NH
Me
NZ
Me
N-Me
Me-NBoc
HN
NHNHBoc
219
175
128
266
366
296
398
416
500
532
570
665
747
838
912
944
989
1046
1077
1168
m/z

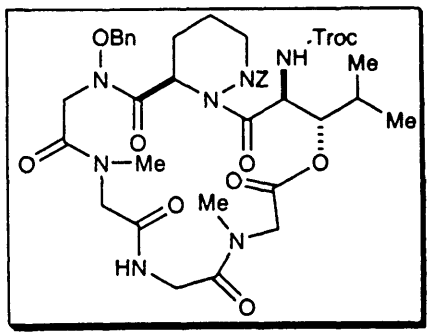


01/02/20 16:04
X: 11 scans, 4.0cm-1

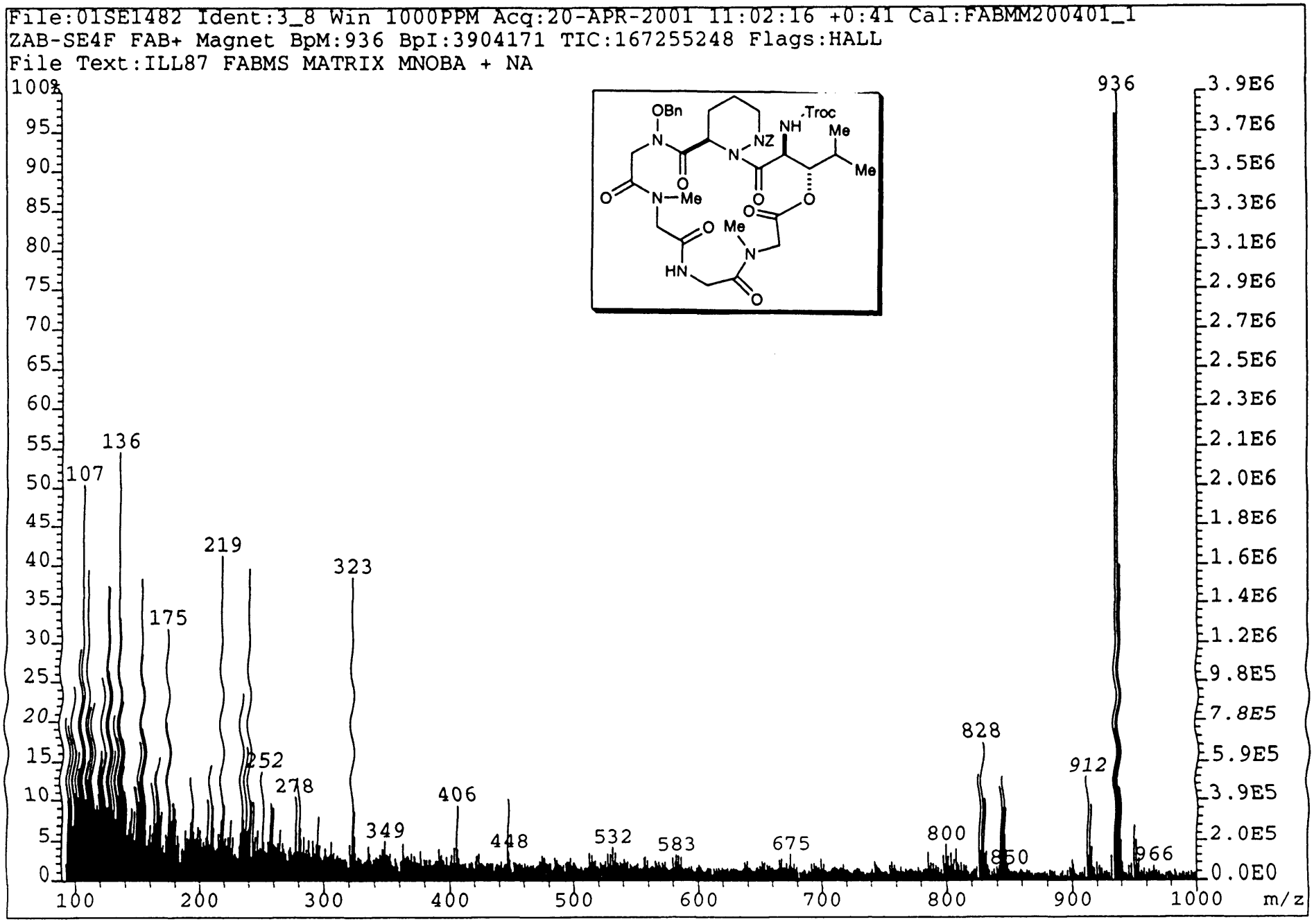
1-LL-87



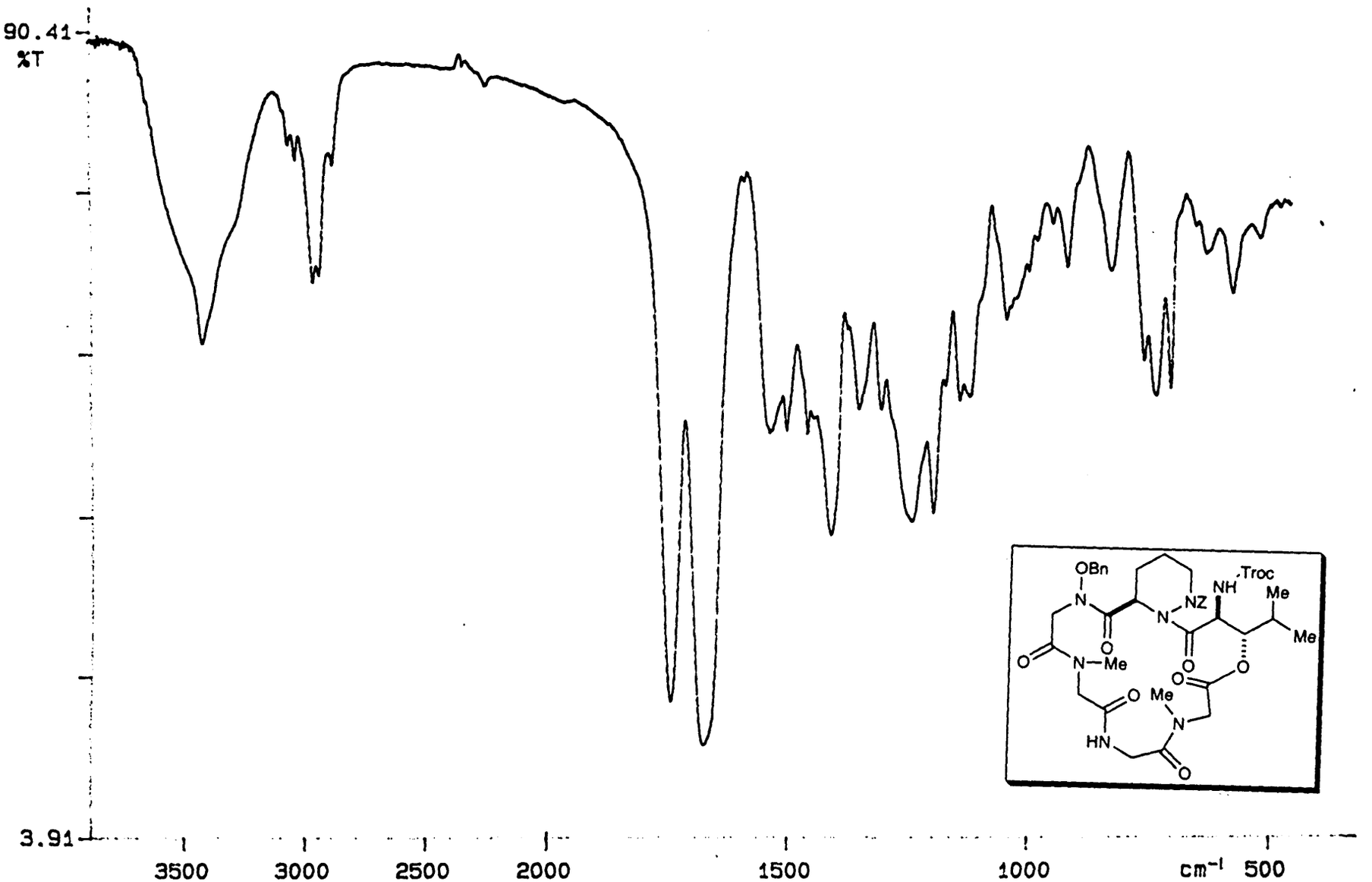
ILL87C


OBn
Troc
NH
Me
NZ
N
Me
O
N–Me
O
O
O
Me
N
HN
O

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

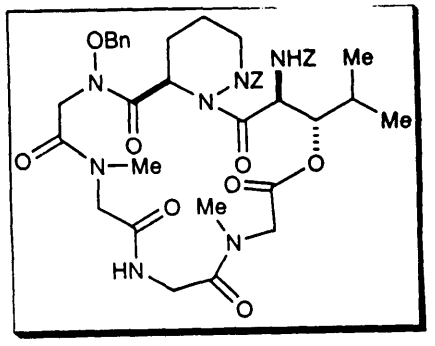

File:01SE1482 Ident:3_8 Win 1000PPM Acq:20-APR-2001 11:02:16 +0:41 Cal:FABMM200401_1
ZAB-SE4F FAB+ Magnet BpM:936 BpI:3904171 TIC:167255248 Flags:HALL
File Text:ILL87 FABMS MATRIX MNOBA + NA
OBn
Troc
NH
NZ
Me
Me
N–Me
Me
HN
936
136
107
219
323
175
828
912
252
278
406
349
448
532
583
675
800
850
966
m/z

PERKIN ELMER

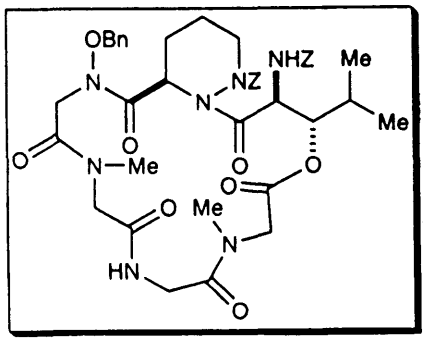


01/02/26 16:39
X: 16 scans, 4.0cm-1

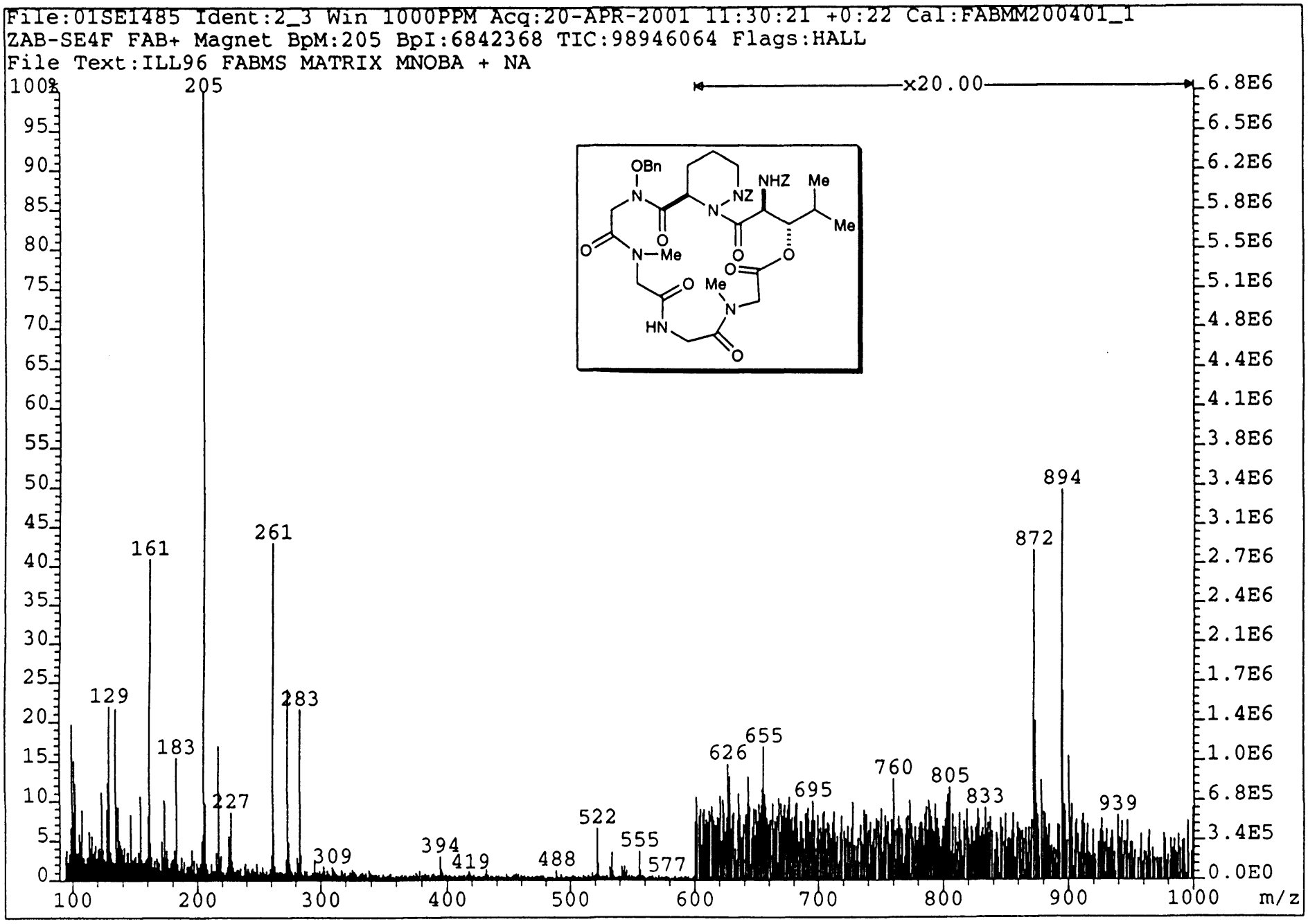
1-LL-96



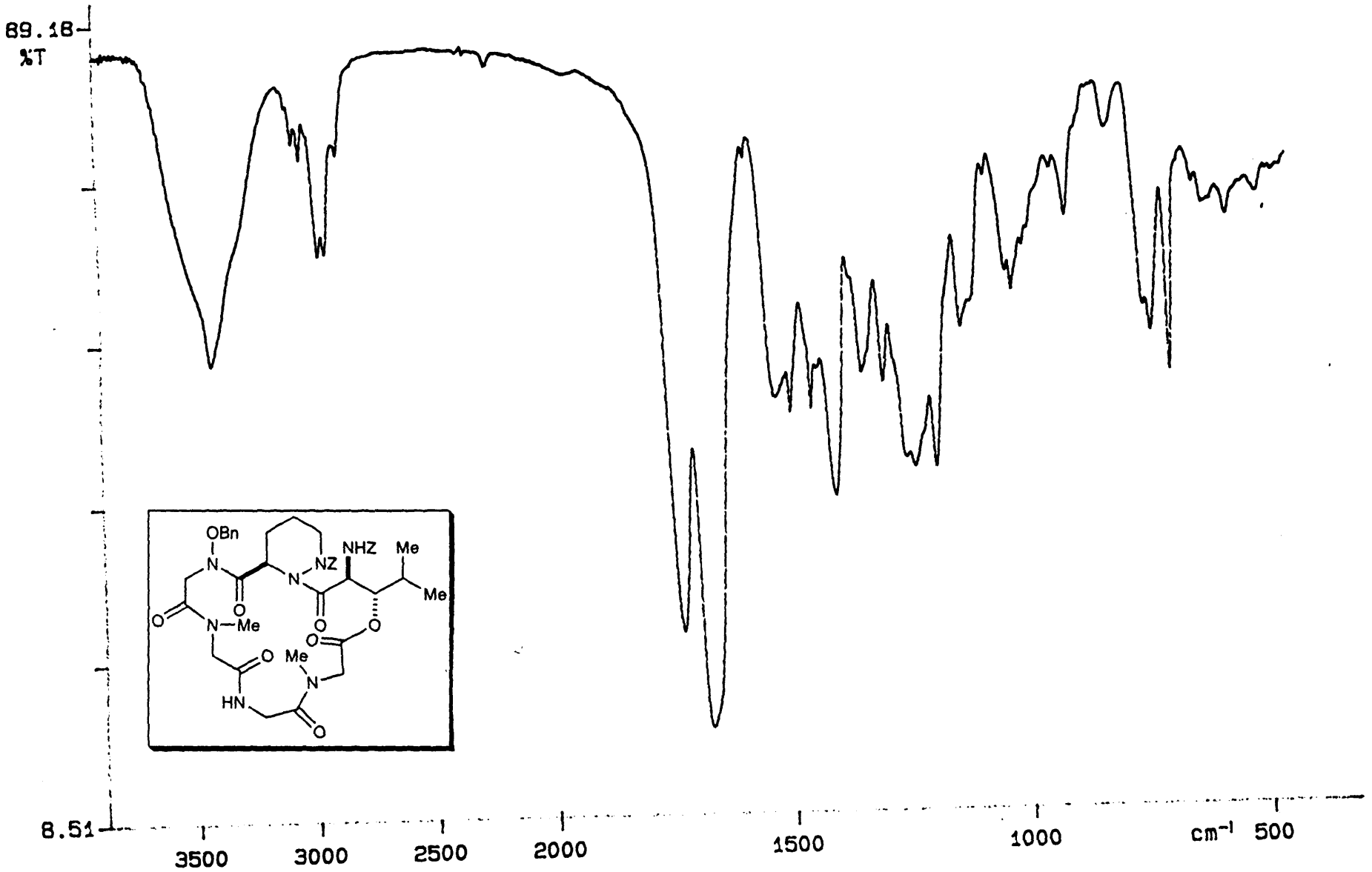
1-LL-96C


OBn
N
NHZ
Me
NZ
N
Me
O
O
N—Me
O
O
O
Me
N
HN
O

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

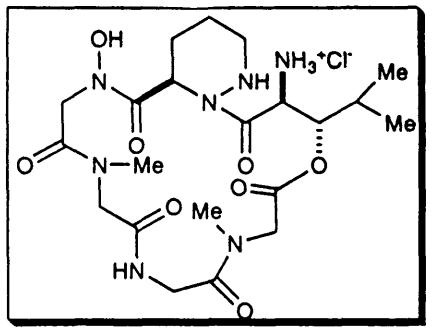

File:01SE1485 Ident:2_3 Win 1000PPM Acq:20-APR-2001 11:30:21 +0:22 Cal:FABMM200401_1
ZAB-SE4F FAB+ Magnet BpM:205 BpI:6842368 TIC:98946064 Flags:HALL
File Text:ILL96 FABMS MATRIX MNOBA + NA
x20.00
OBn
NHZ
Me
NZ
Me
N-Me
Me
HN
205
161
261
129
283
183
227
309
394
419
488
522
555
577
626
655
695
760
805
833
872
894
939
m/z

PERKIN ELMER

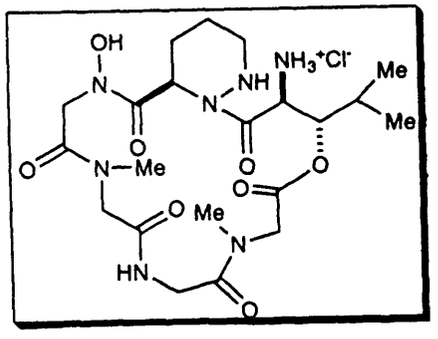


01/02/26 17:55
X: 16 scans, 4.0cm-1

1-LL-125

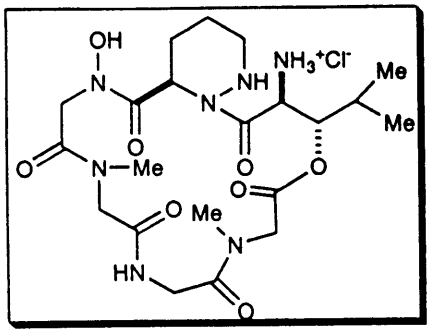


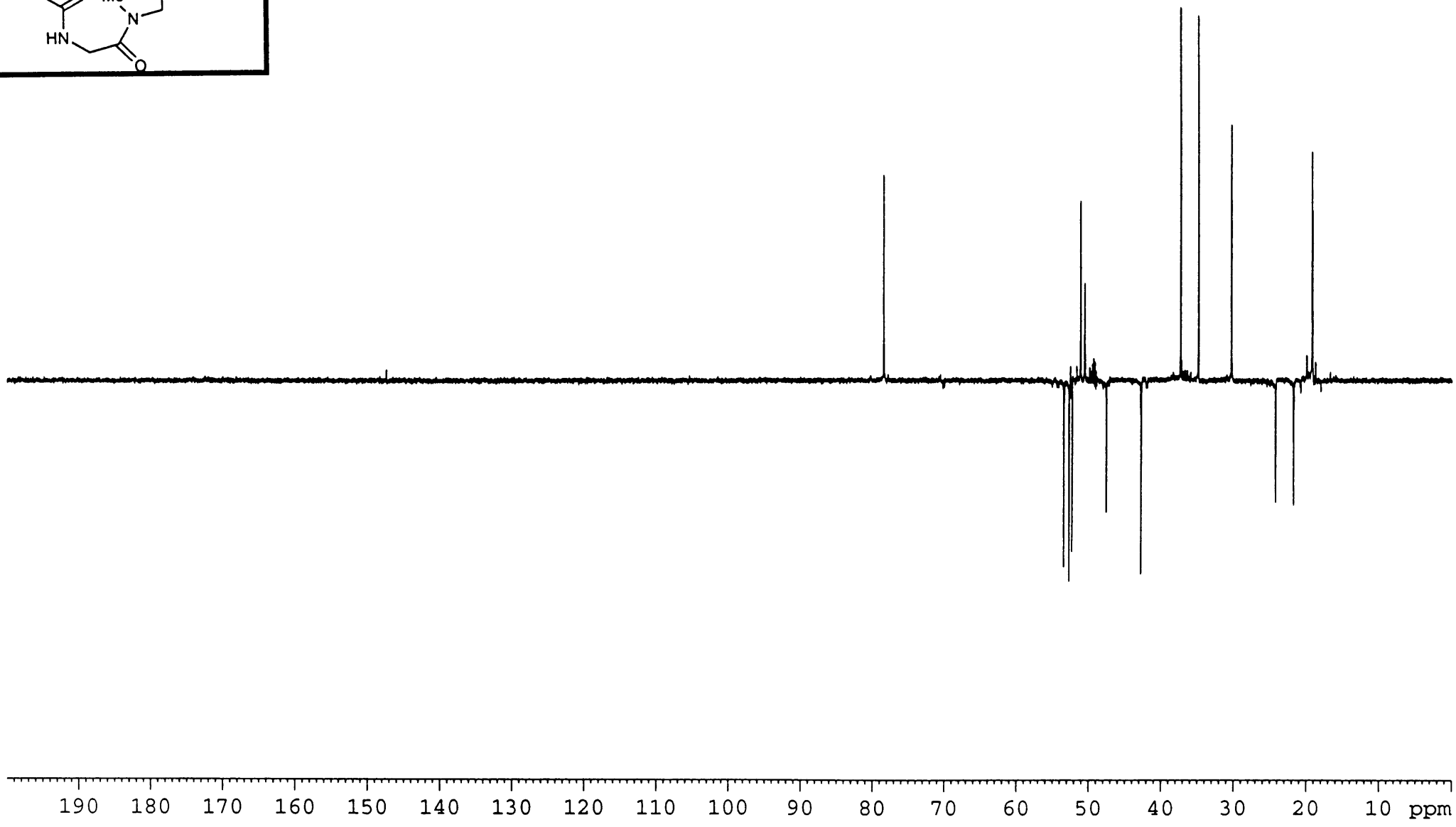
ILL125

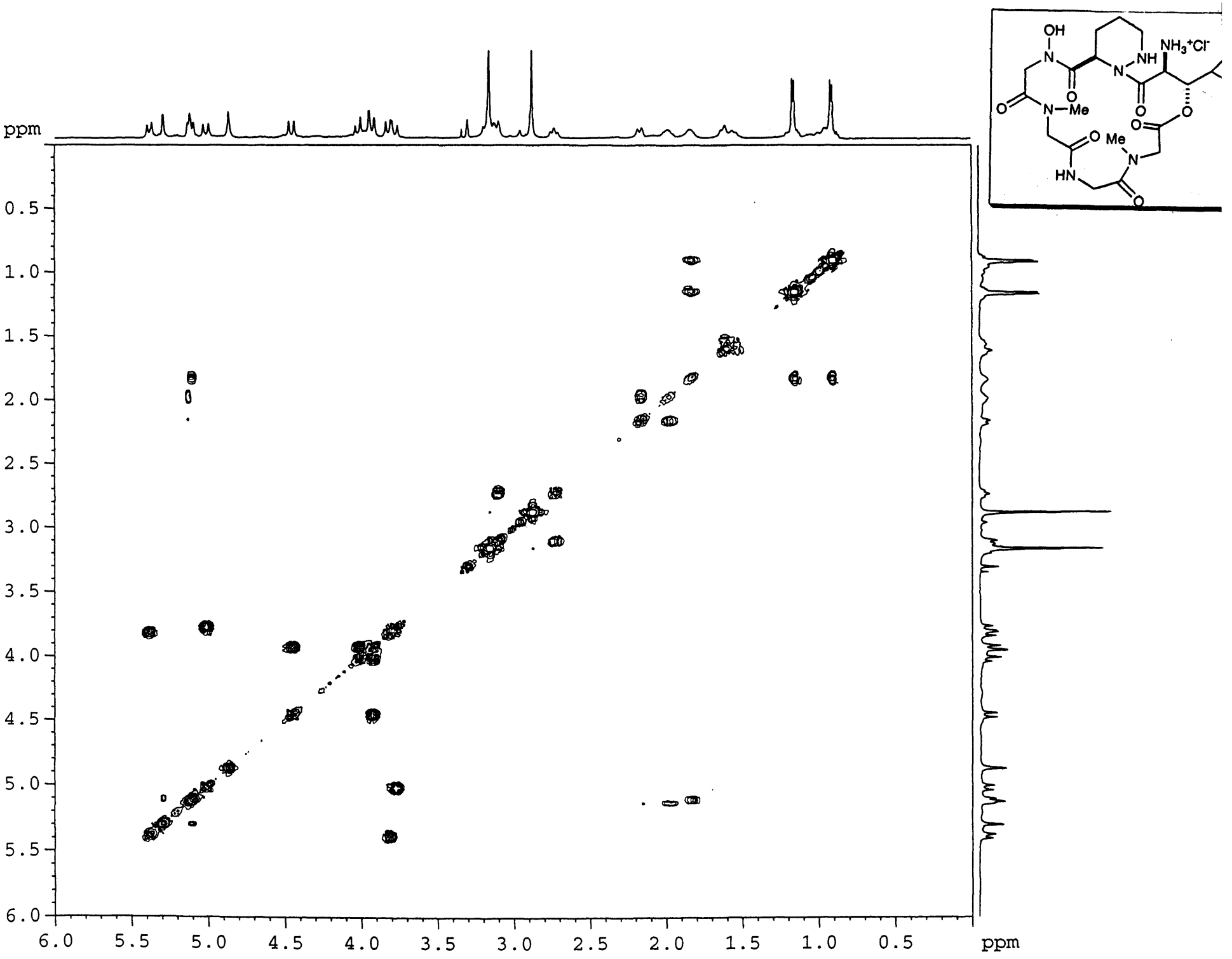

OH
N
NH
NH3+Cl-
Me
Me
N
O
O
N–Me
O
O
O
Me
N
O
HN
O

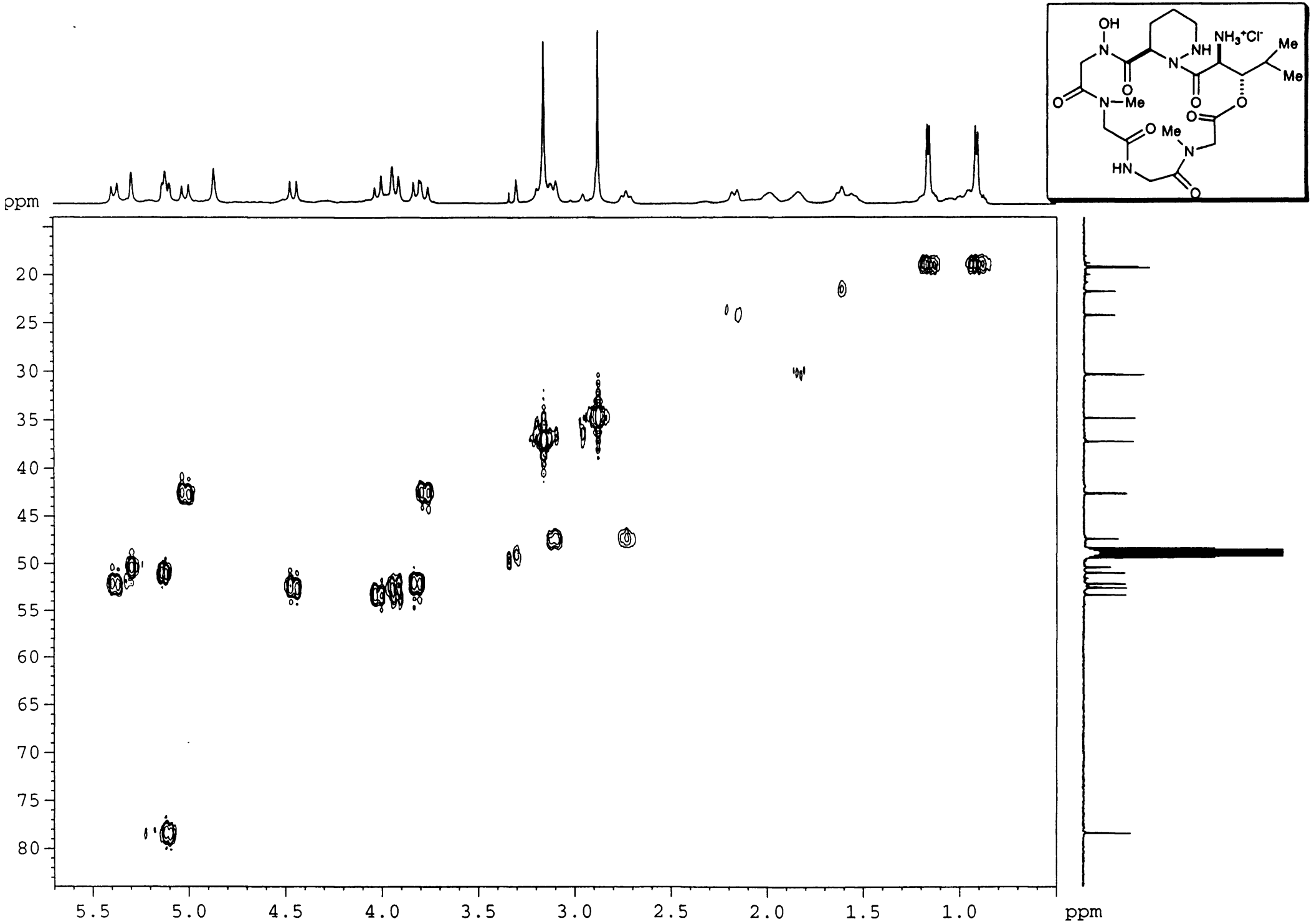
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

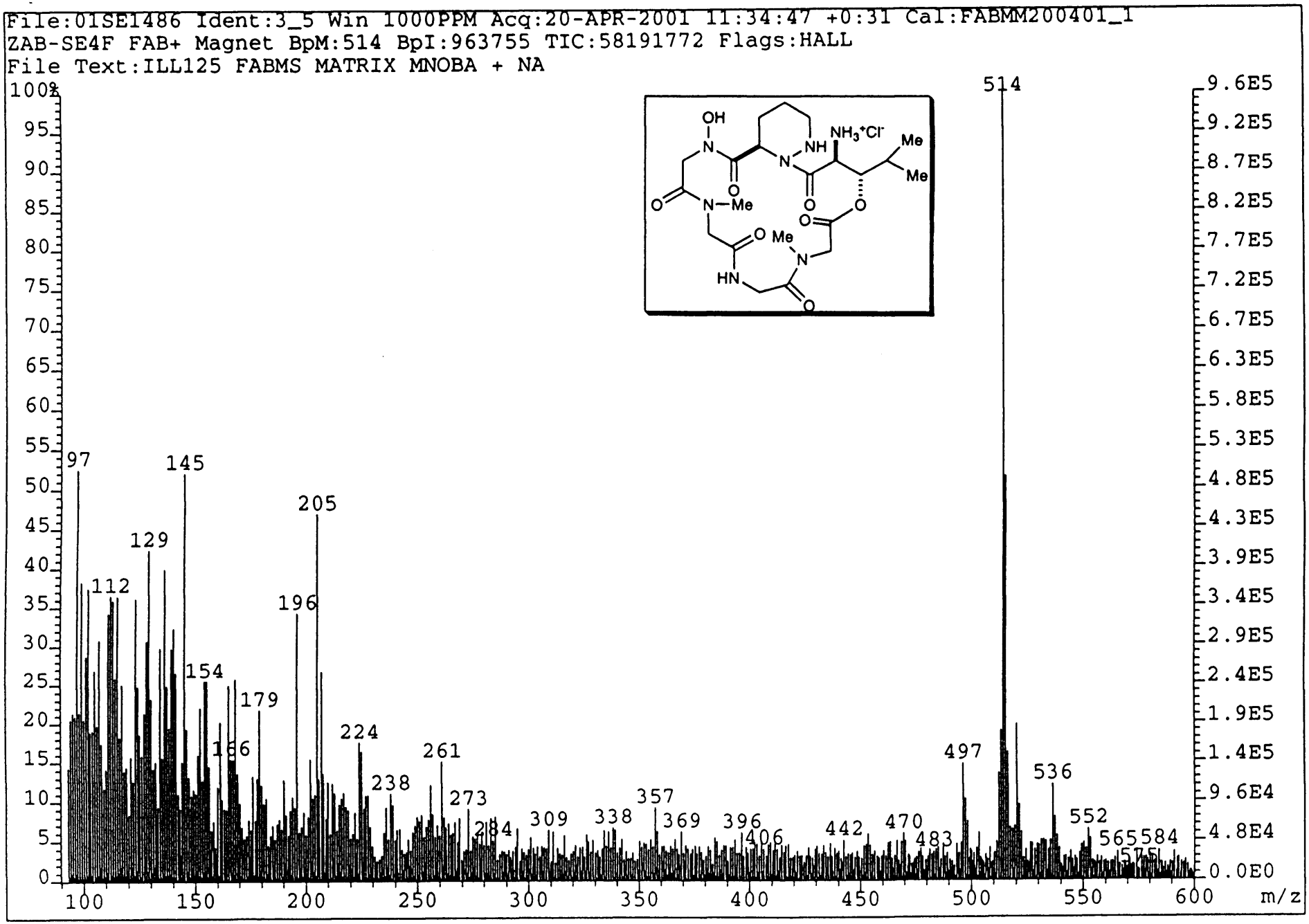
ILL125

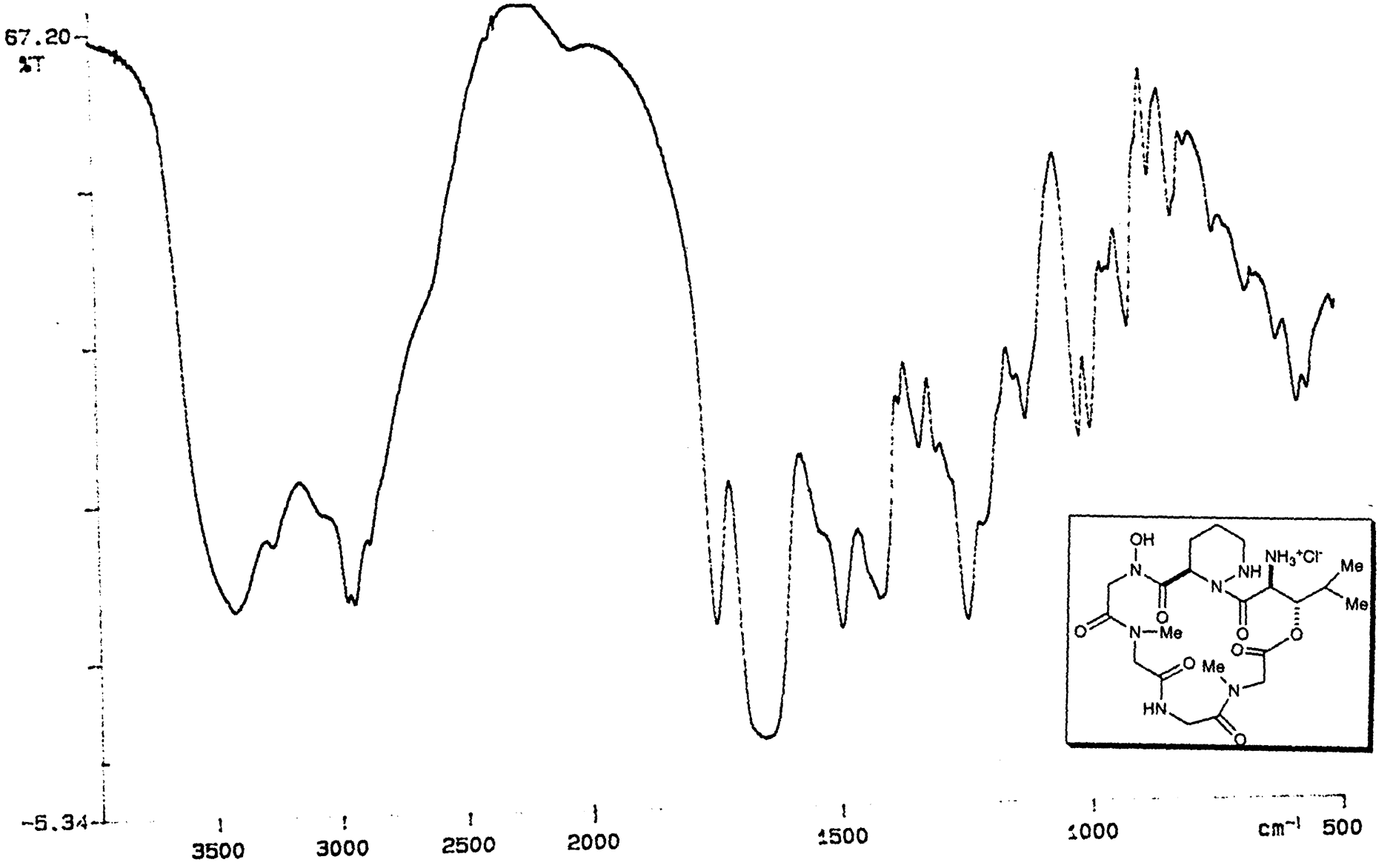

OH
N
NH
$NH_3^+Cl^-$
Me
Me
O
O
N–Me
O
O
O
O
Me
N
HN
O


190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
OH
N
NH
NH3+Cl-
O
N–Me
Me
HN

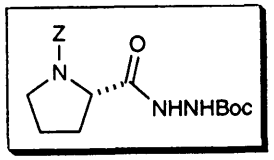

ppm
OH
NH3+Cl-
Me
Me
NH
N
N
O
O
O
O
O
N–Me
O
Me
N
HN
O
20
25
30
35
40
45
50
55
60
65
70
75
80
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm


File:01SE1486 Ident:3_5 Win 1000PPM Acq:20-APR-2001 11:34:47 +0:31 Cal:FABMM200401_1
ZAB-SE4F FAB+ Magnet BpM:514 BpI:963755 TIC:58191772 Flags:HALL
File Text:ILL125 FABMS MATRIX MNOBA + NA
514
97
145
205
129
112
196
154
179
224
166
261
238
273
357
309
338
369
396
406
442
470
483
497
536
552
565
584
575
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
9.6E5
9.2E5
8.7E5
8.2E5
7.7E5
7.2E5
6.7E5
6.3E5
5.8E5
5.3E5
4.8E5
4.3E5
3.9E5
3.4E5
2.9E5
2.4E5
1.9E5
1.4E5
9.6E4
4.8E4
0.0E0
100
150
200
250
300
350
400
450
500
550
600
m/z
OH
NH3+Cl-
NH
Me
Me
N-Me
Me
HN

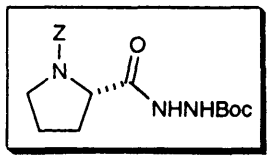


01/04/19 13:11
N: 16 scans. 4.0cm-1. apod none

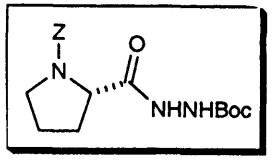
I-LL-184
In CDCl3 298K



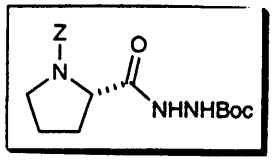
I-LL-184
In CDCl3 298K

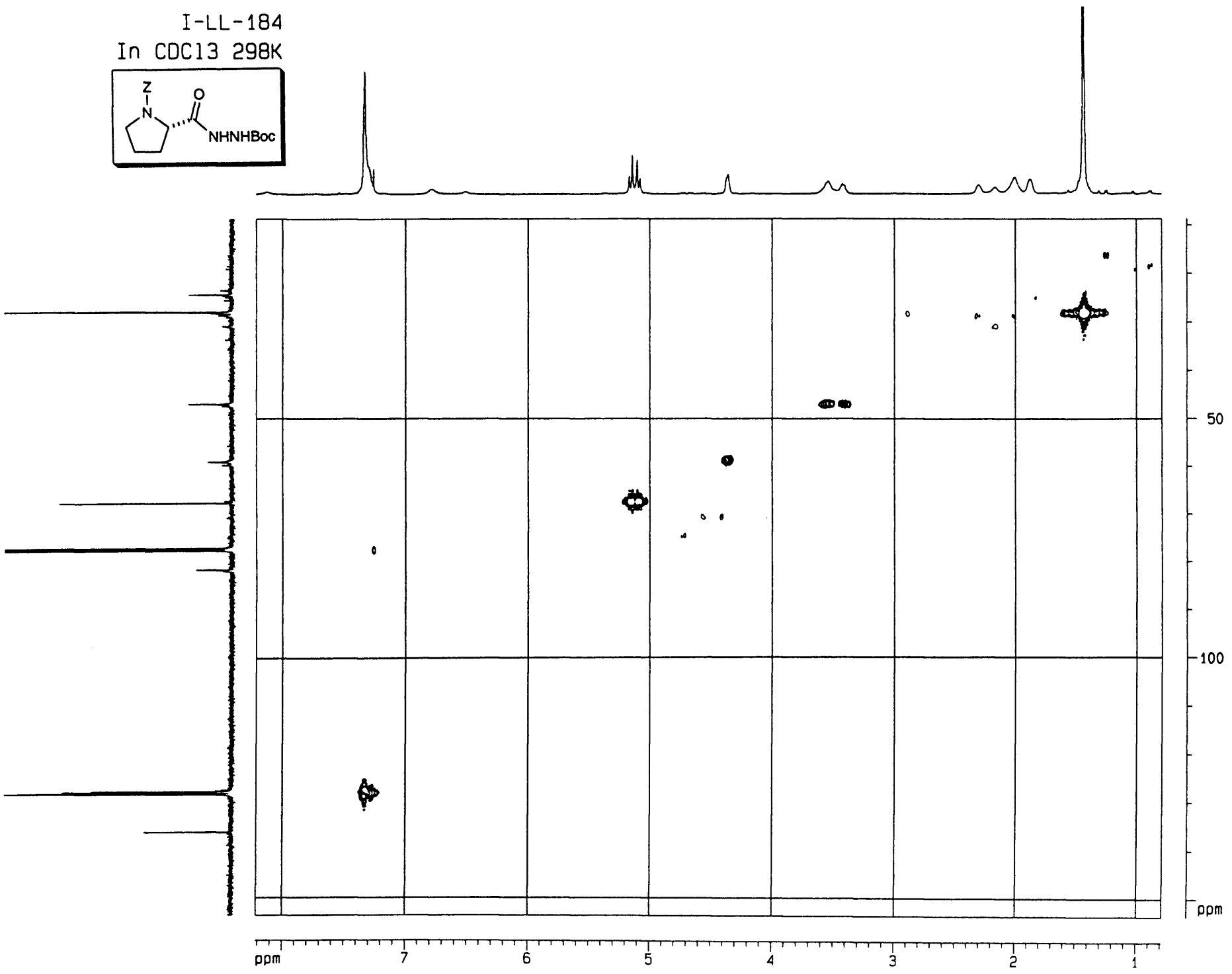


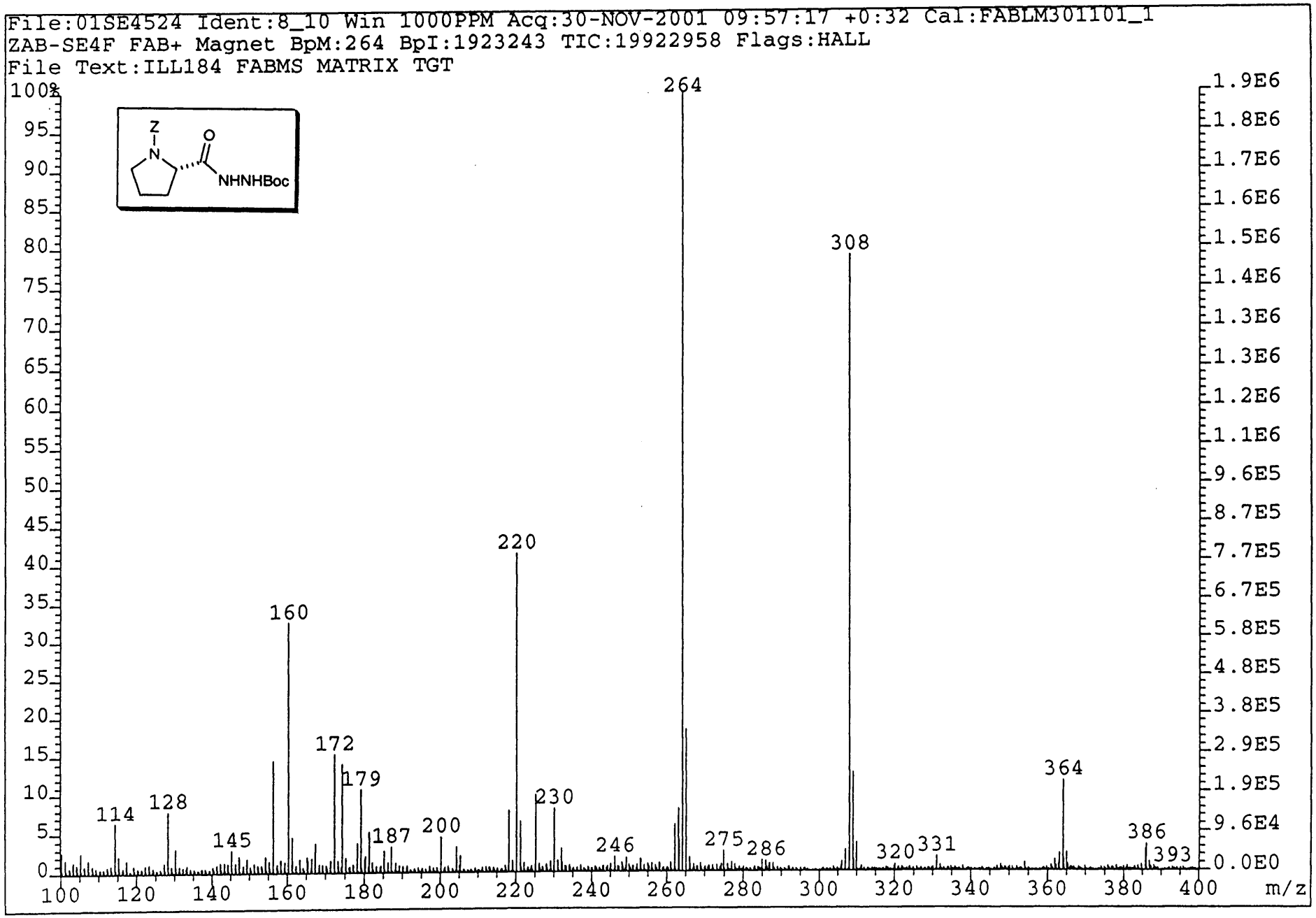
I-LL-184
In CDCl3 298K



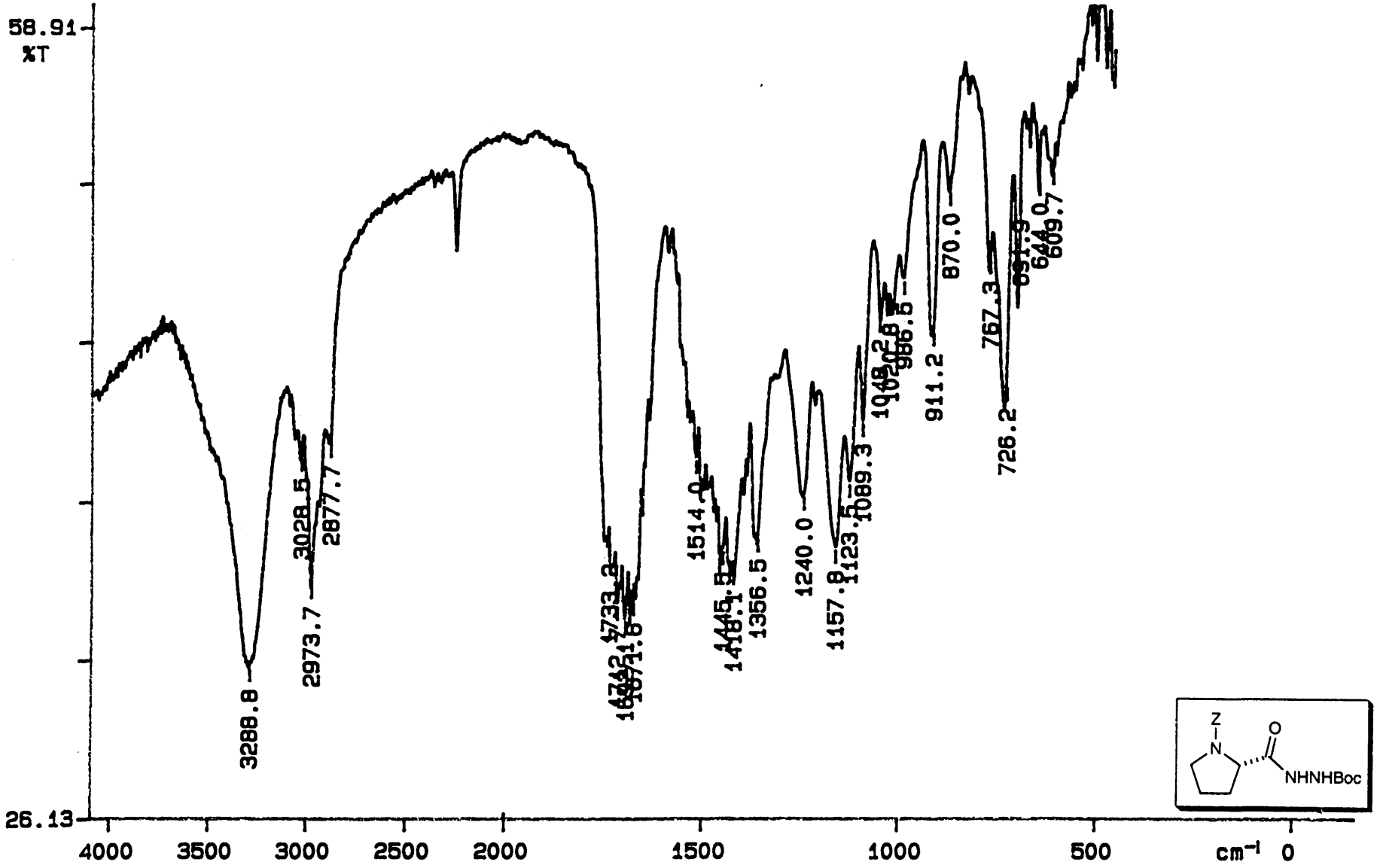
I-LL-184
In CDCl3 298K




I-LL-184
In CDCl3 298K
Z
N
O
NHNHBoc
50
100
ppm
ppm
7
6
5
4
3
2
1

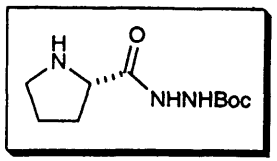

File:01SE4524 Ident:8_10 Win 1000PPM Acq:30-NOV-2001 09:57:17 +0:32 Cal:FABLM301101_1
ZAB-SE4F FAB+ Magnet BpM:264 BpI:1923243 TIC:19922958 Flags:HALL
File Text:ILL184 FABMS MATRIX TGT
Z
N
O
NHNHBoc
264
308
220
160
172
179
364
230
114
128
145
187
200
246
275
286
320
331
386
393
m/z

PERKIN ELMER

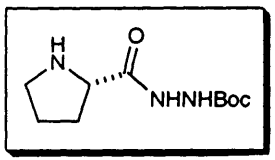


02/03/20 12:38
X: 16 scans, 4.0cm-1

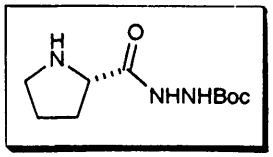
I-LL-187
In CDCl3 298K



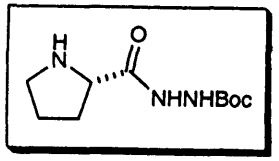
I-LL-187
In CDCl3 298K

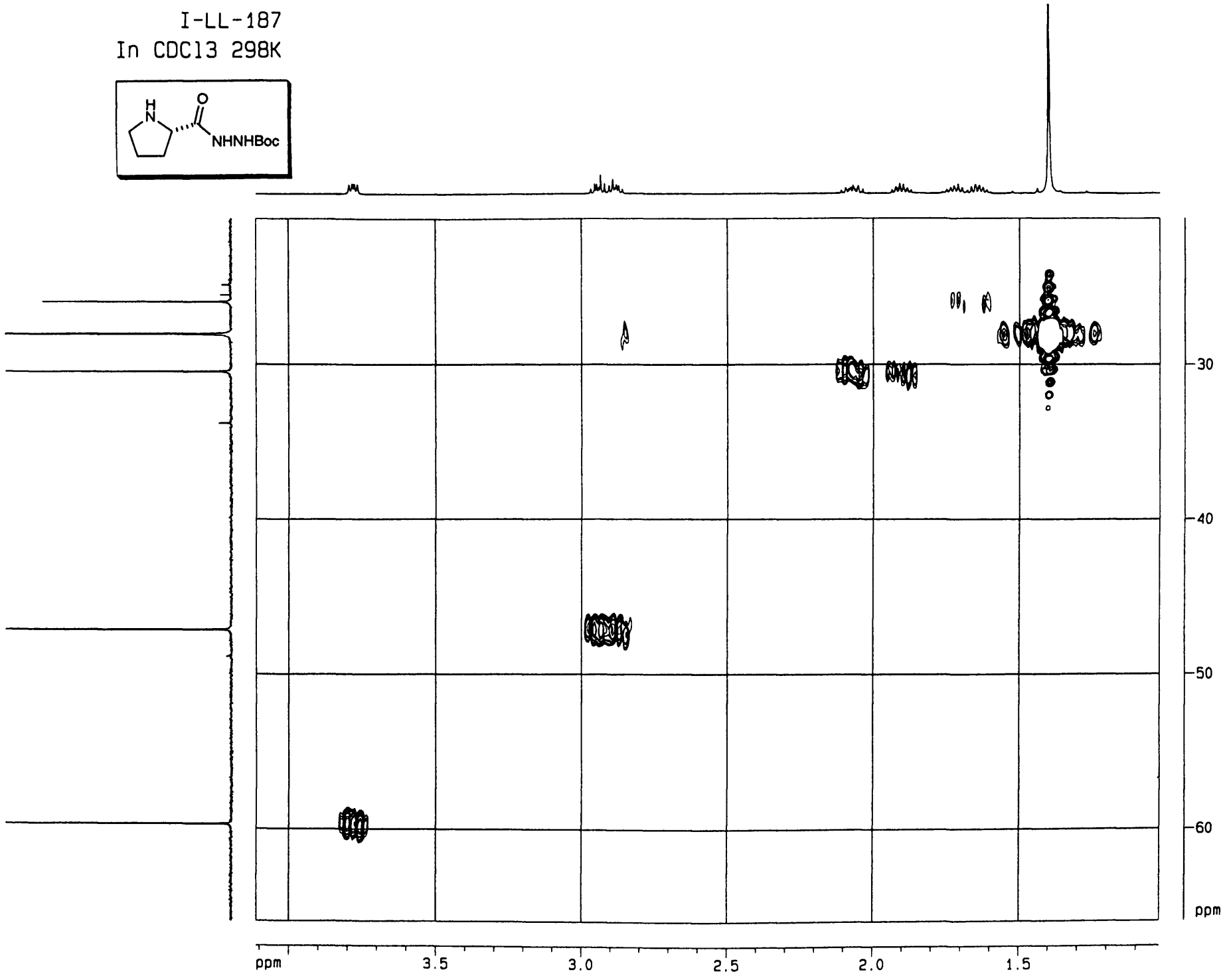


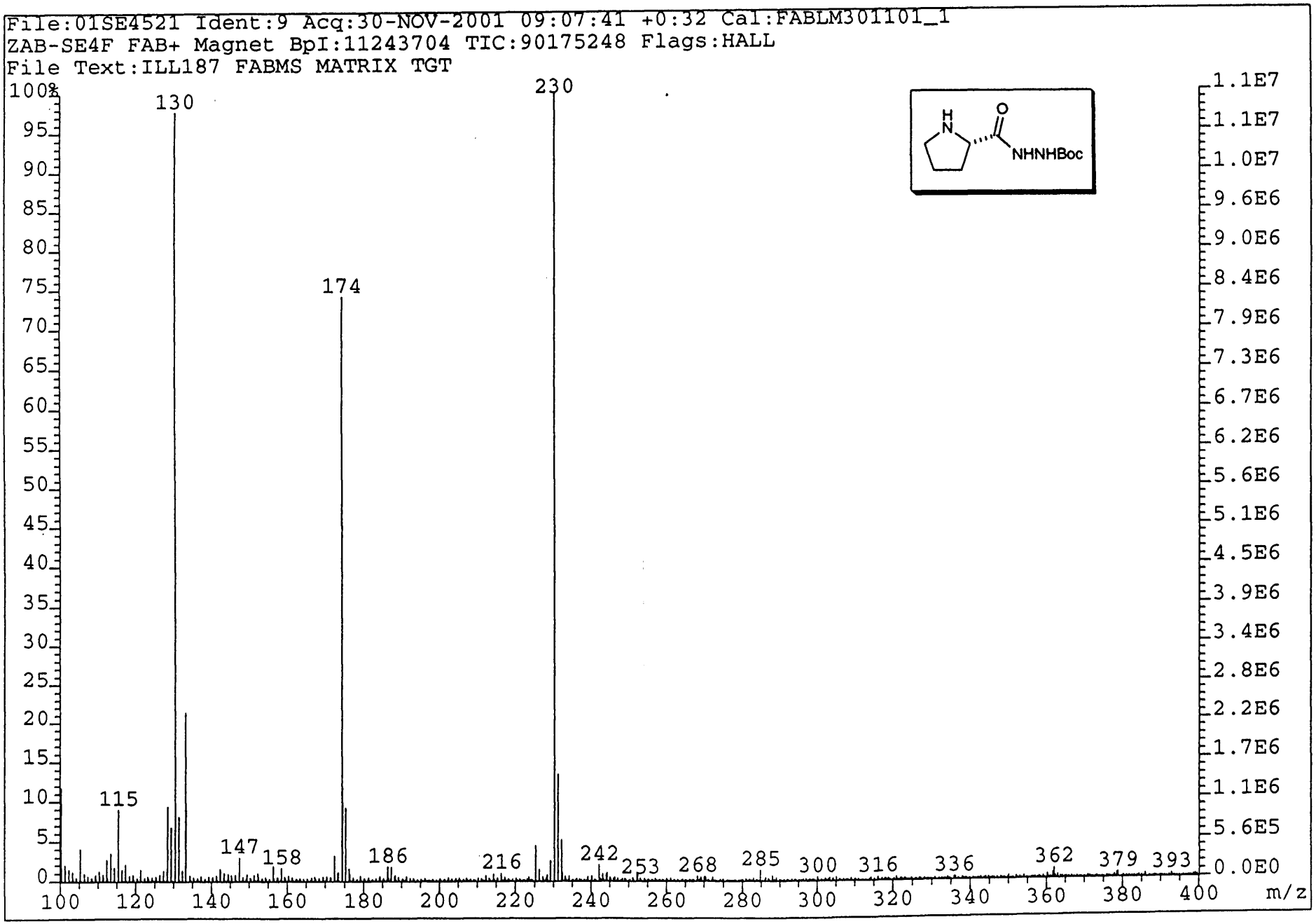
I-LL-187
In CDCl3 298K



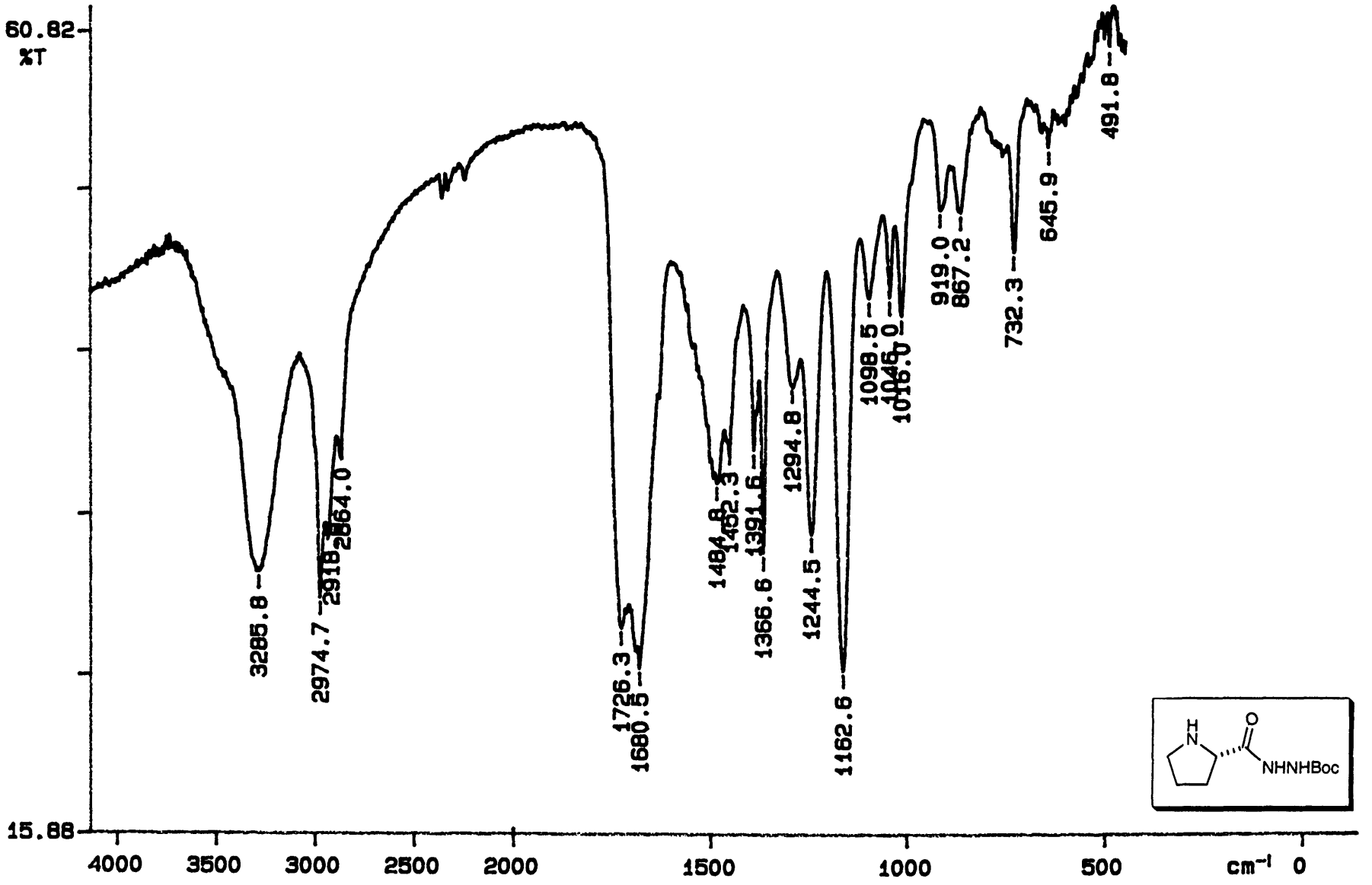
I-LL-187
In CDCl3 298K




I-LL-187
In CDCl3 298K
NHNHBoc
30
40
50
60
ppm
ppm
3.5
3.0
2.5
2.0
1.5

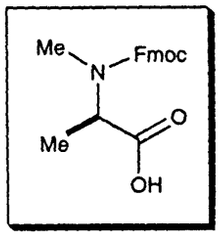

File:01SE4521 Ident:9 Acq:30-NOV-2001 09:07:41 +0:32 Cal:FABLM301101_1
ZAB-SE4F FAB+ Magnet BpI:11243704 TIC:90175248 Flags:HALL
File Text:ILL187 FABMS MATRIX TGT
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
130
230
174
115
147
158
186
216
242
253
268
285
300
316
336
362
379
393
NHNHBoc
1.1E7
1.1E7
1.0E7
9.6E6
9.0E6
8.4E6
7.9E6
7.3E6
6.7E6
6.2E6
5.6E6
5.1E6
4.5E6
3.9E6
3.4E6
2.8E6
2.2E6
1.7E6
1.1E6
5.6E5
0.0E0
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
m/z

PERKIN ELMER

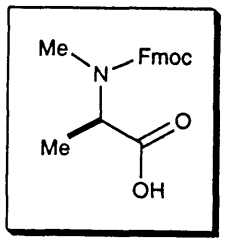


02/03/19 15:28
X: 16 scans, 4.0cm-1

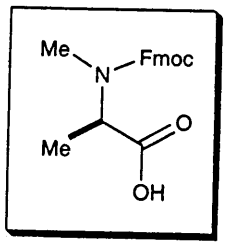
I-LL-185
In CDCl3 298K

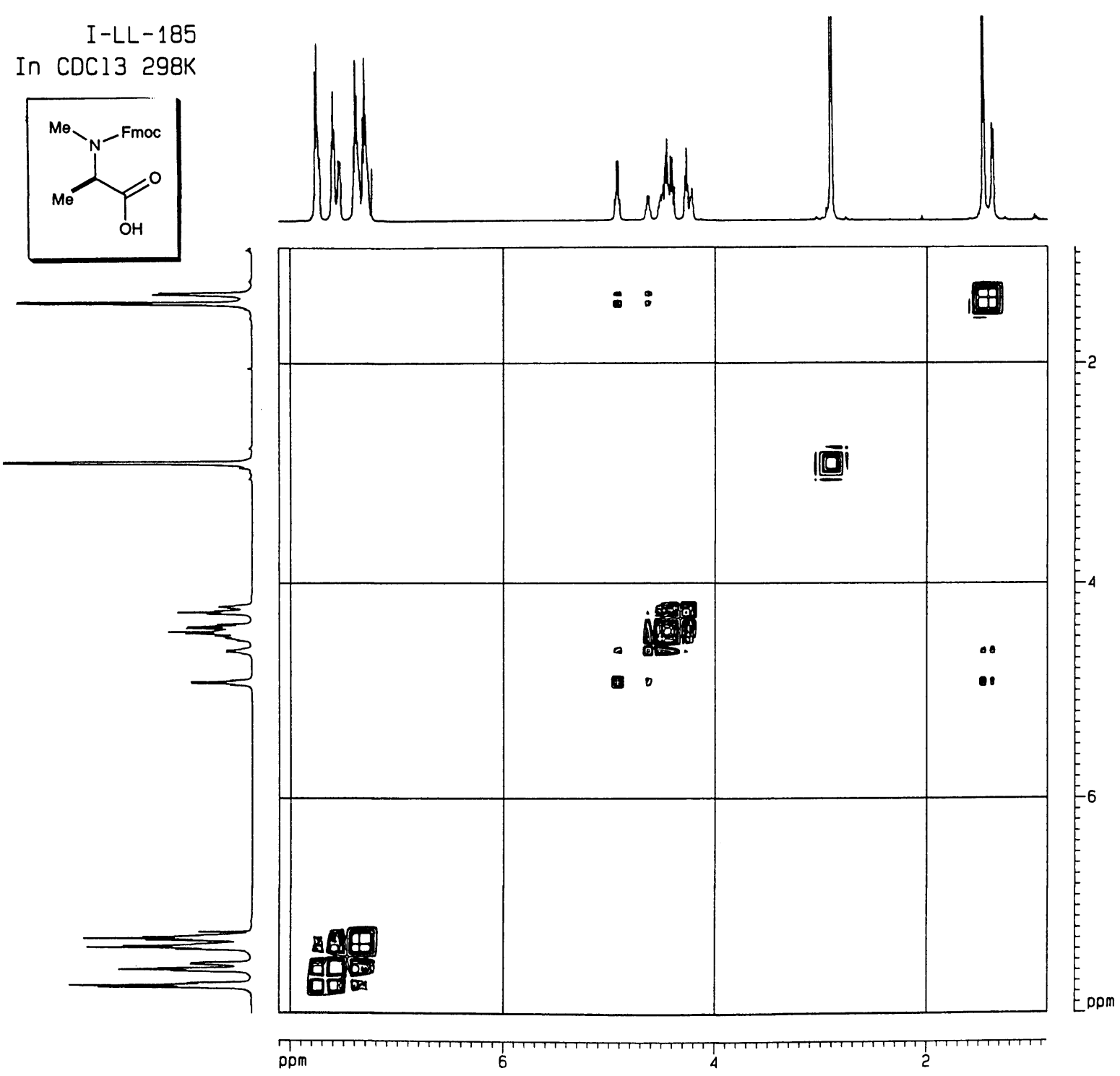


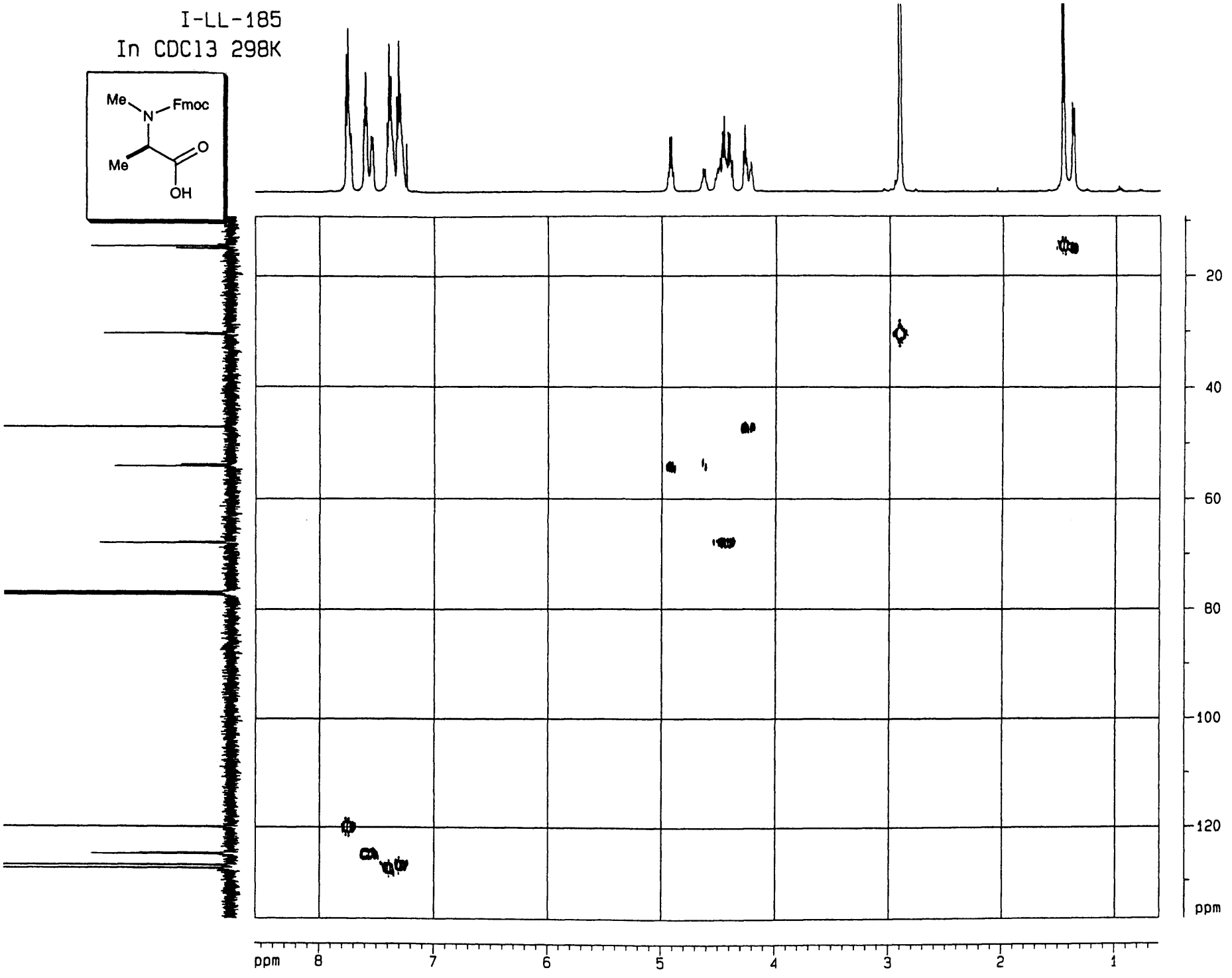
I-LL-185
In CDCl3 298K

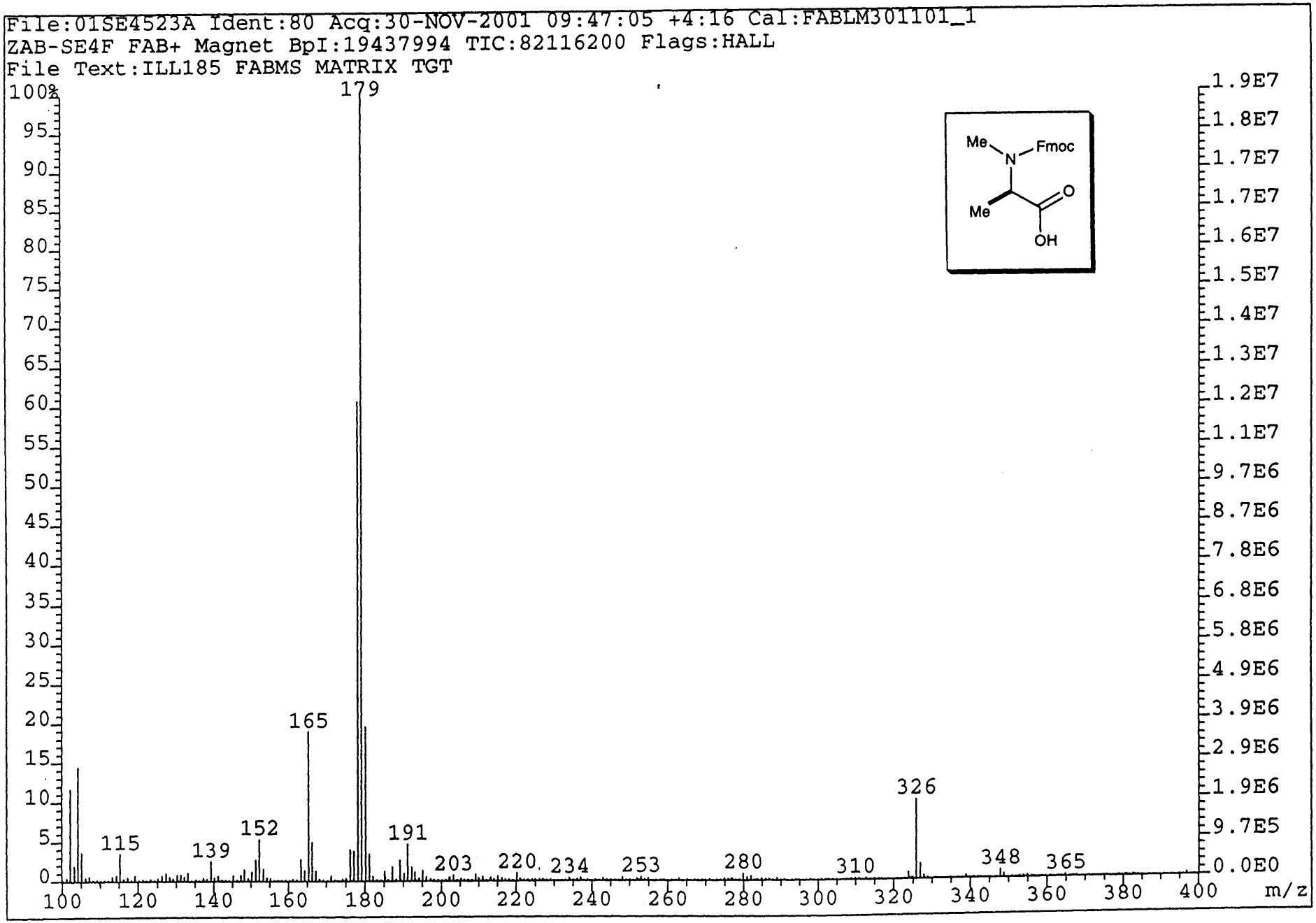


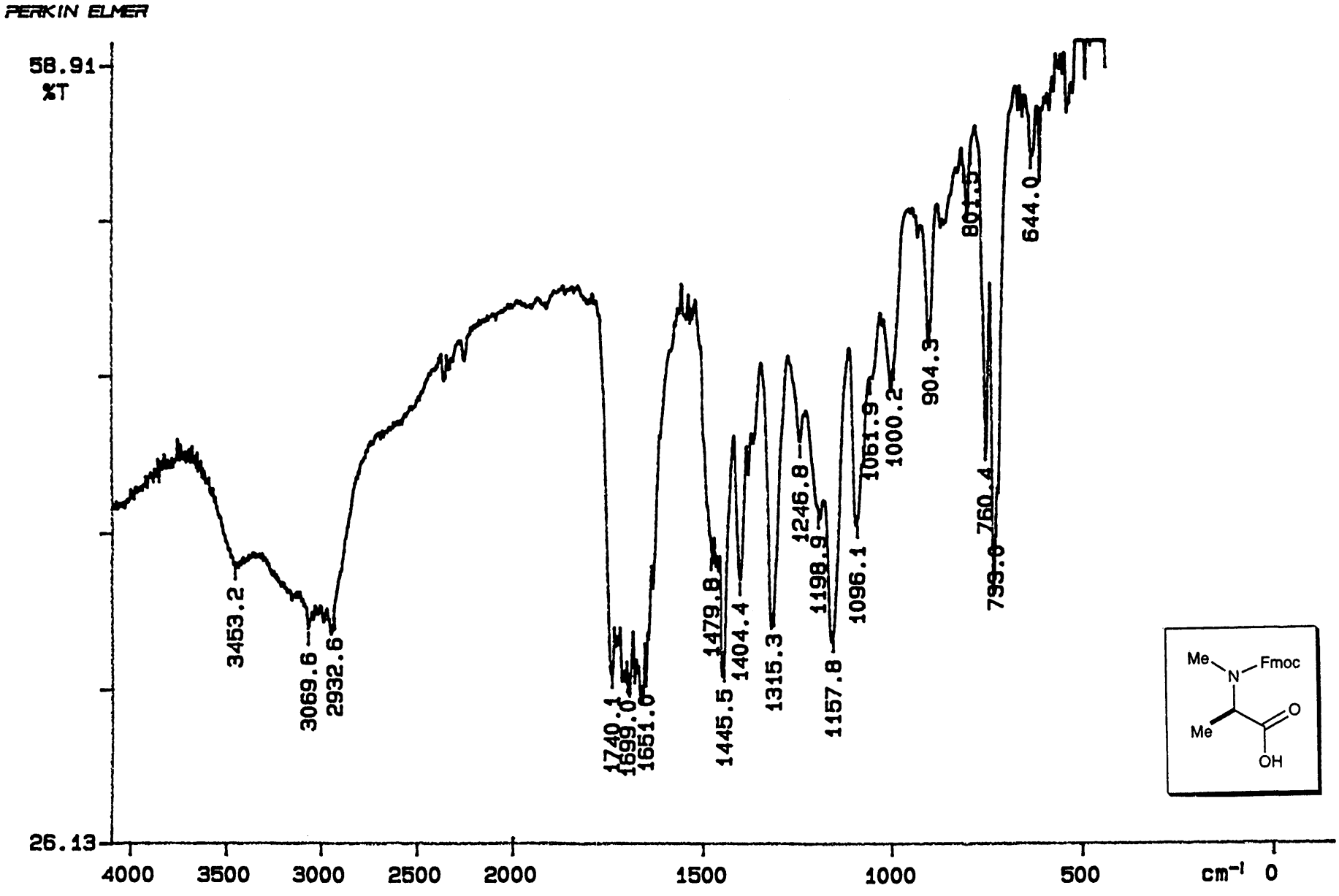
I-LL-185
In CDCl3 298K




I-LL-185
In CDCl3 298K
Me
N
Fmoc
Me
O
OH
ppm
6
4
2
ppm

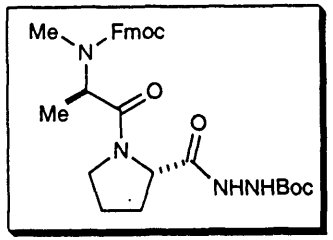

I-LL-185
In CDCl3 298K
Me
N
Fmoc
O
Me
OH
20
40
60
80
100
120
ppm
ppm
8
7
6
5
4
3
2
1


File:01SE4523A Ident:80 Acq:30-NOV-2001 09:47:05 +4:16 Cal:FABLM301101_1
ZAB-SE4F FAB+ Magnet BpI:19437994 TIC:82116200 Flags:HALL
File Text:ILL185 FABMS MATRIX TGT
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
1.9E7
1.8E7
1.7E7
1.7E7
1.6E7
1.5E7
1.4E7
1.3E7
1.2E7
1.1E7
9.7E6
8.7E6
7.8E6
6.8E6
5.8E6
4.9E6
3.9E6
2.9E6
1.9E6
9.7E5
0.0E0
179
165
326
115
139
152
191
203
220
234
253
280
310
348
365
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
m/z
Me
N
Fmoc
Me
O
OH

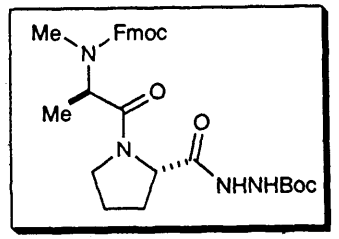


02/03/19 15: 40
X: 16 scans, 4.0cm-1

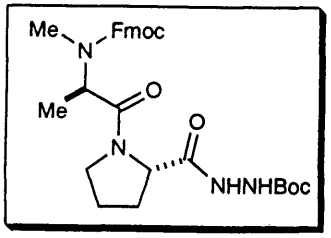
III-LL-18
In CDCl3 298K



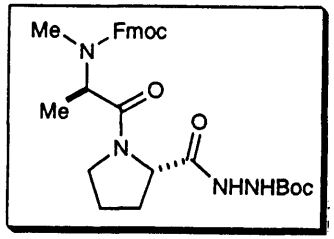
III-LL-18
In CDCl3 298K



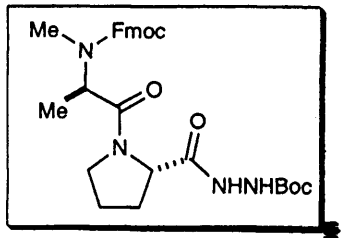
III-LL-18
In CDCl3 298K

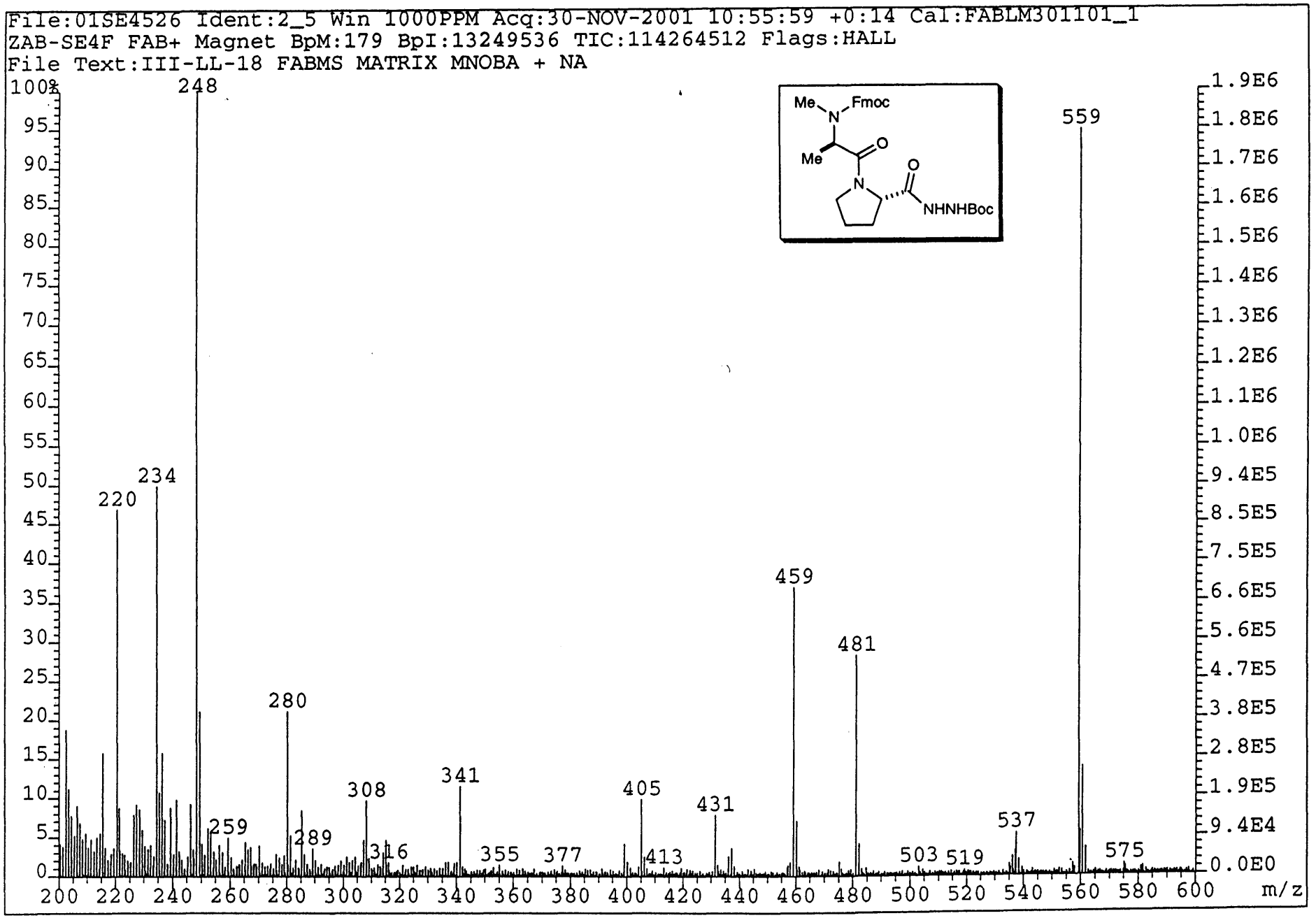


III-LL-18
In CDCl3 298K

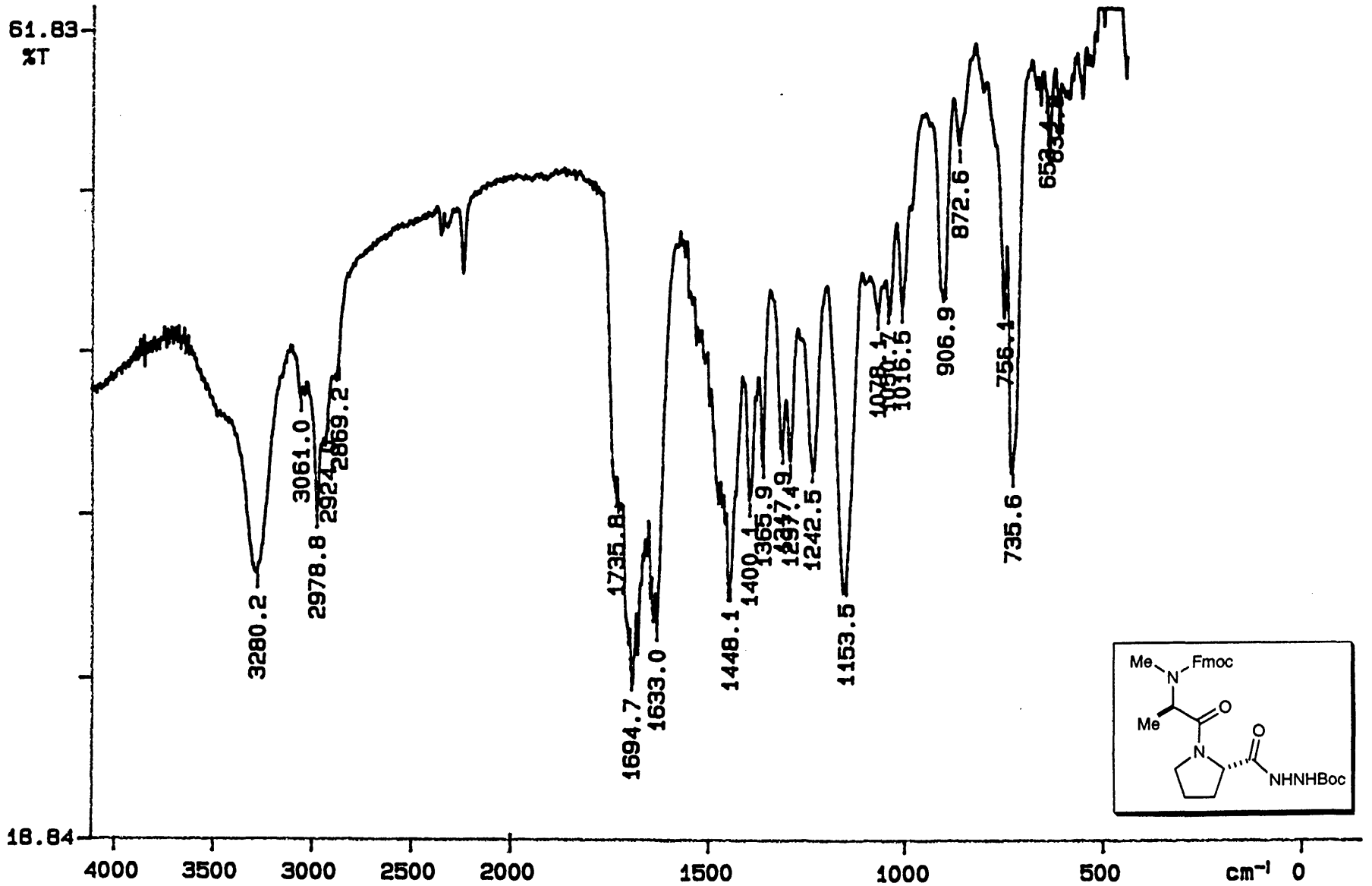


III-LL-18
In CDCl3 298K



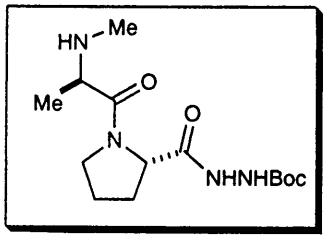

File:01SE4526 Ident:2_5 Win 1000PPM Acq:30-NOV-2001 10:55:59 +0:14 Cal:FABLM301101_1
ZAB-SE4F FAB+ Magnet BpM:179 BpI:13249536 TIC:114264512 Flags:HALL
File Text:III-LL-18 FABMS MATRIX MNOBA + NA
Me
N
Fmoc
Me
O
N
O
NHNHBoc
248
559
234
220
459
481
280
341
308
405
431
259
289
316
355
377
413
503
519
537
575
m/z

PERKIN ELMER

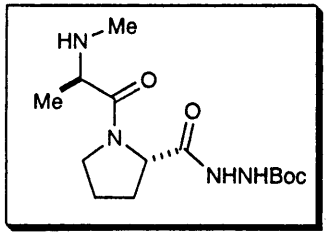


02/03/20 12:54
X: 16 scans, 4.0cm-1

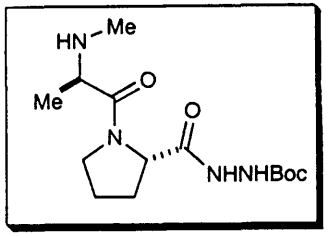
III-LL-19
In CDCl3 298K



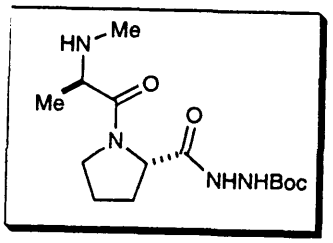
III-LL-19
In CDCl3 298K



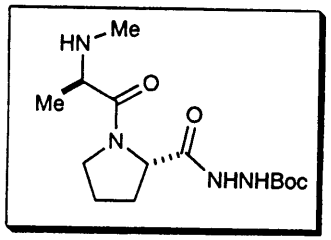
III-LL-19
In CDCl3 298K



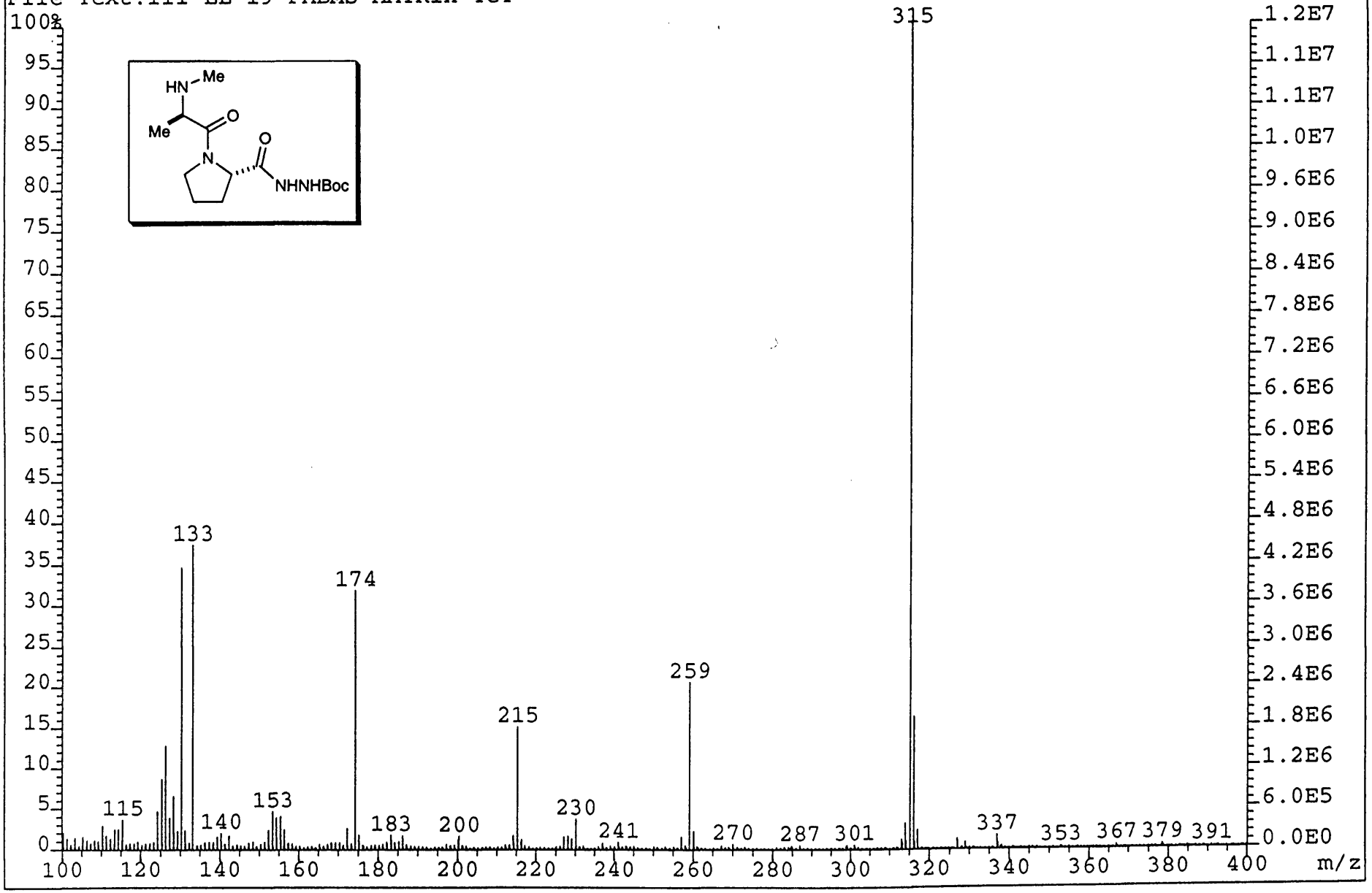
III-LL-19
In CDCl3 298K



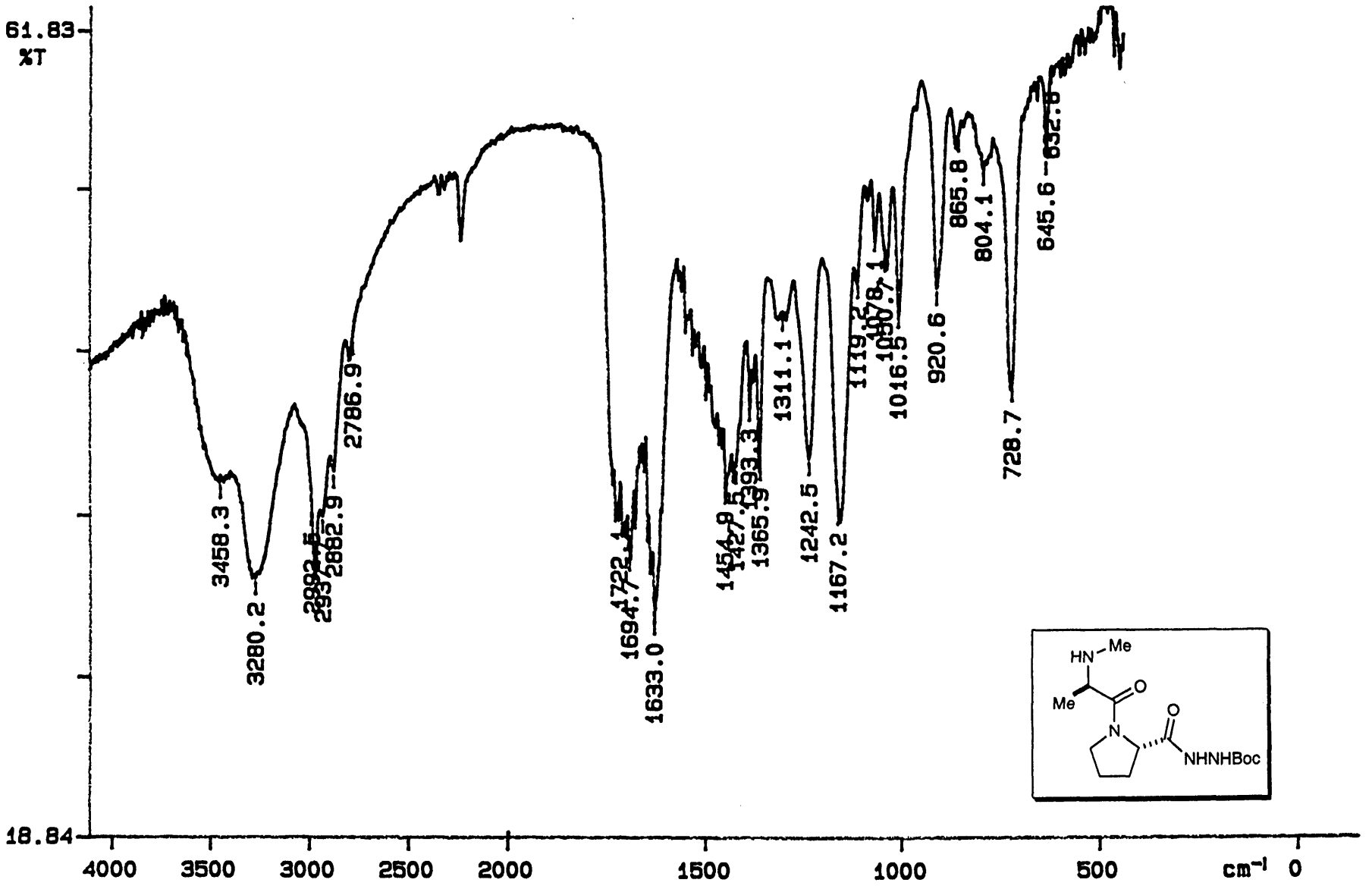
III-LL-19
In CDCl3 298K



File:01SE4522 Ident:37_53 Win 1000PPM Acq:30-NOV-2001 09:27:52 +2:26 Cal:FABLM301101_1
ZAB-SE4F FAB+ Magnet BpM:315 BpI:12010195 TIC:88281040 Flags:HALL
File Text:III-LL-19 FABMS MATRIX TGT

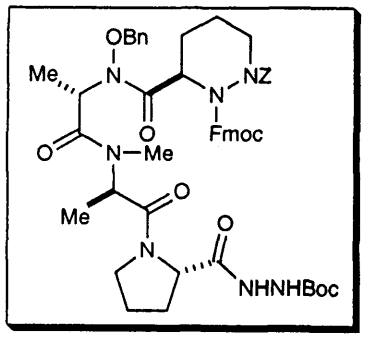


PERKIN ELMER

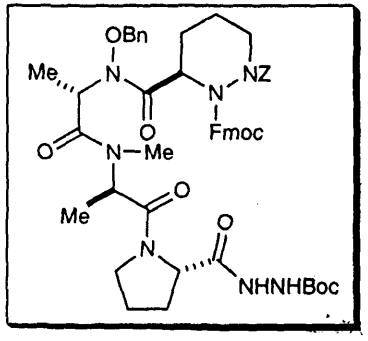


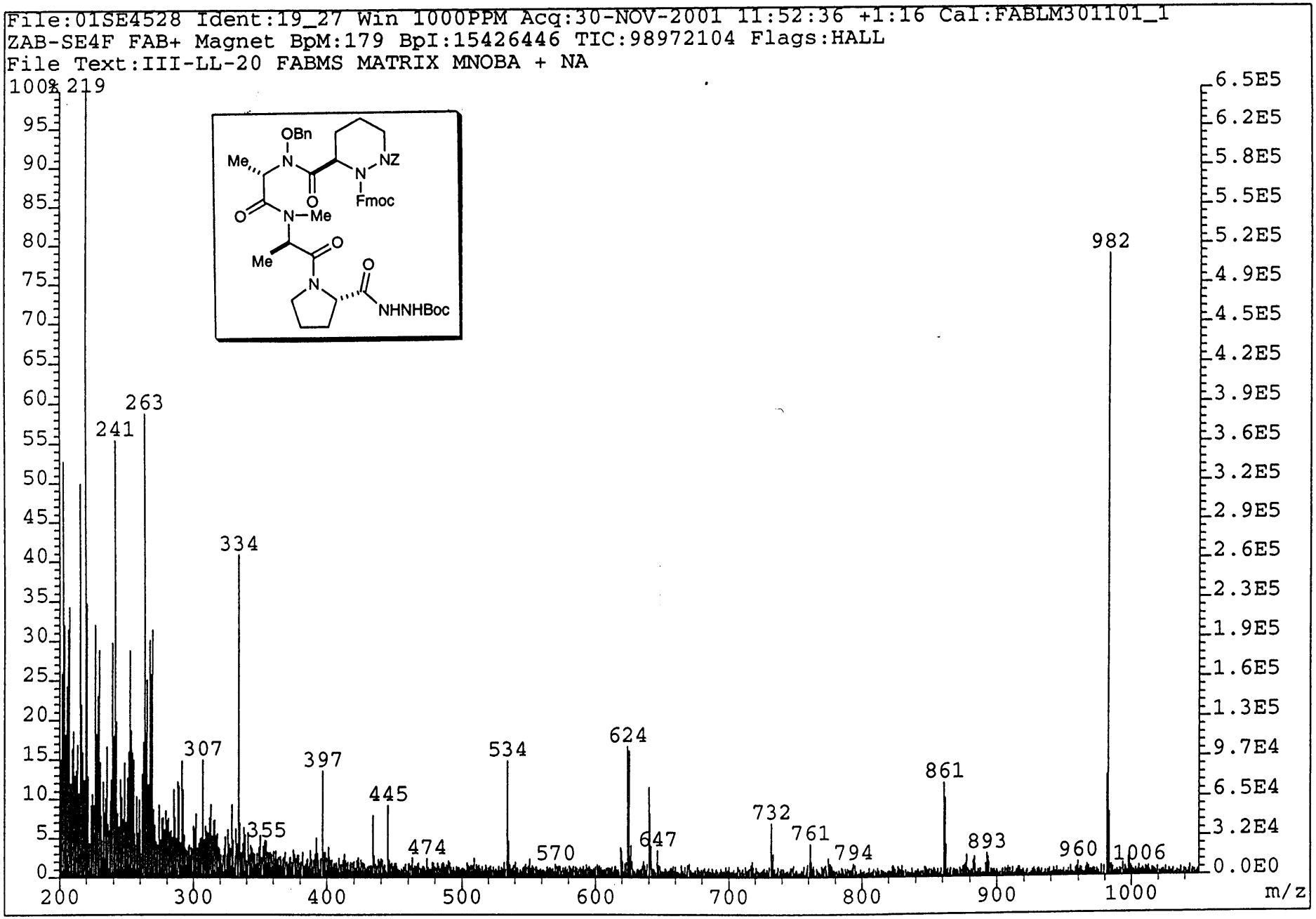
02/03/20 13: 15
X: 16 scans, 4.0cm-1

III-LL-20
In CDCl3 298K

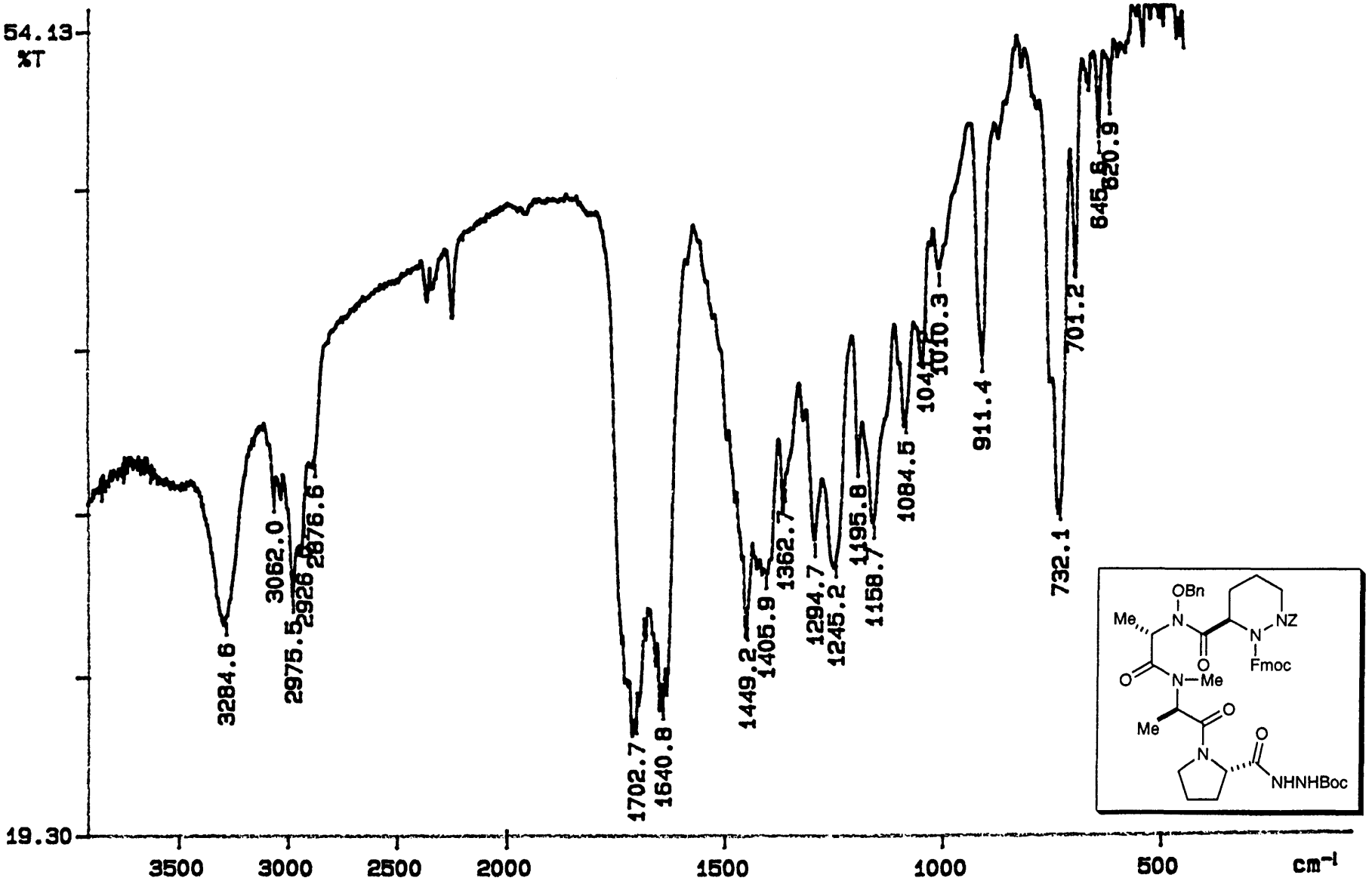


III-LL-20
In CDCl3 298K



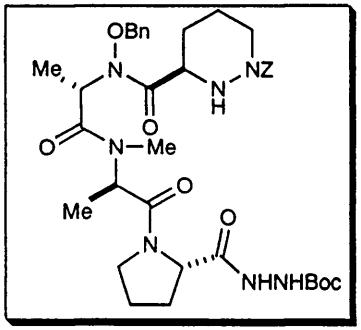

File:01SE4528 Ident:19_27 Win 1000PPM Acq:30-NOV-2001 11:52:36 +1:16 Cal:FABLM301101_1
ZAB-SE4F FAB+ Magnet BpM:179 BpI:15426446 TIC:98972104 Flags:HALL
File Text:III-LL-20 FABMS MATRIX MNOBA + NA
100% 219
263
241
334
307
355
397
445
474
534
570
624
647
732
761
794
861
893
960
982
1006
OBn
Me
N
NZ
Fmoc
O
N—Me
Me
NHNHBoc
m/z

PERKIN ELMER

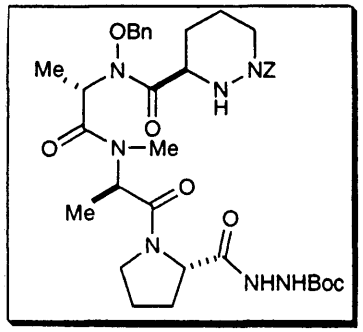


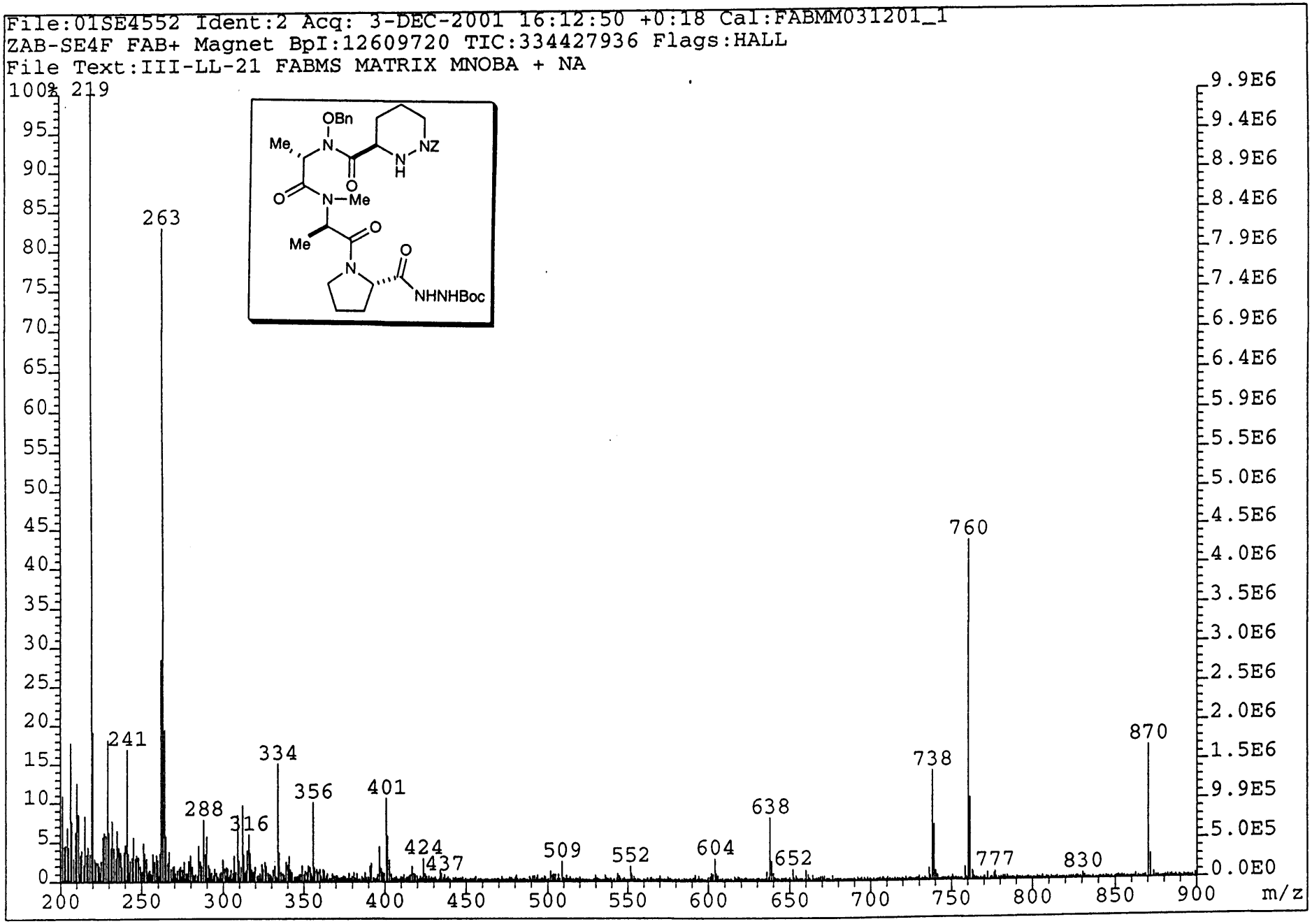
02/03/21 14:03
X: 16 scans, 4.0cm-1

III-LL-21
In CDCl3 298K

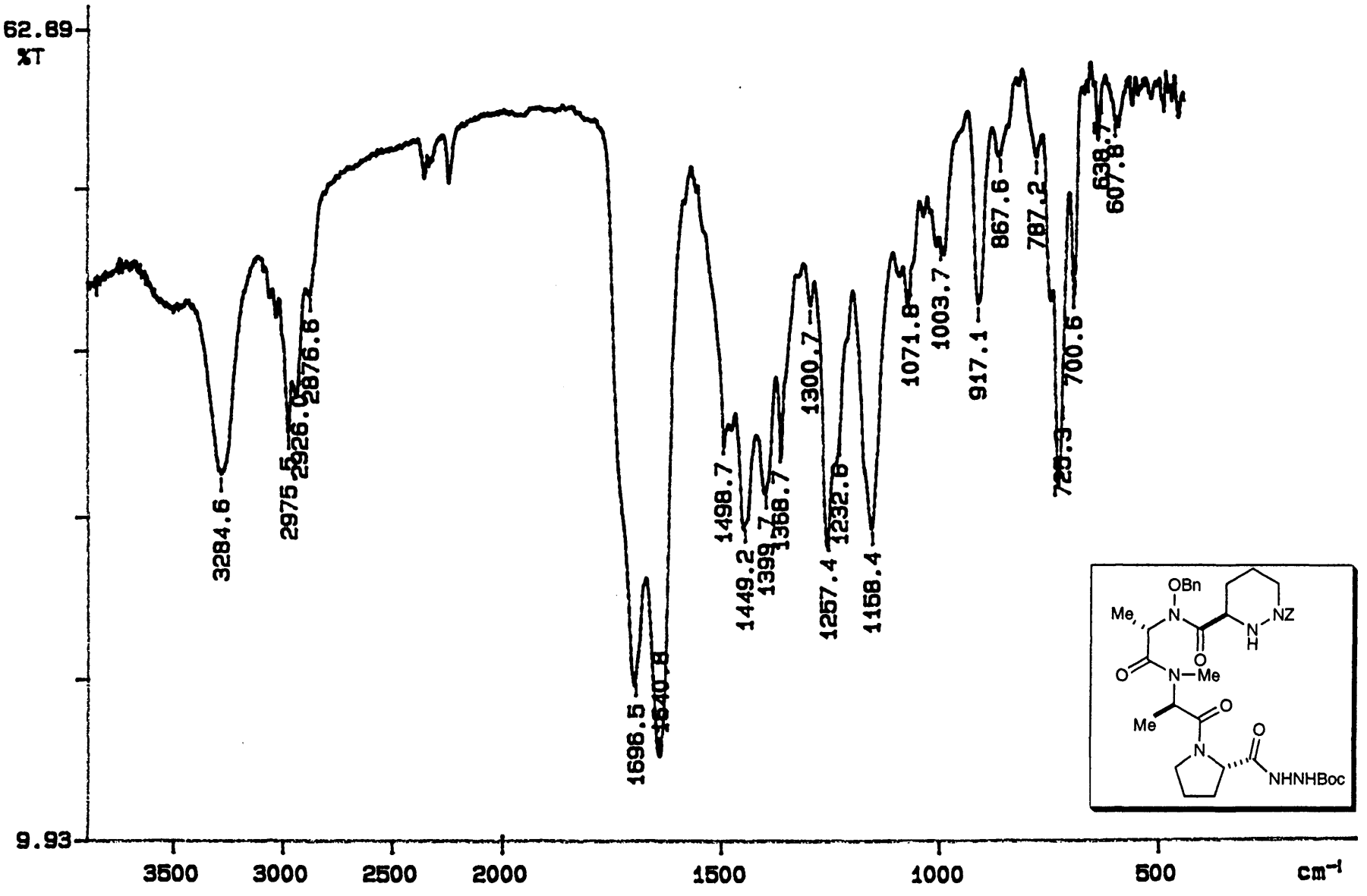


III-LL-21
In CDCl3 298K



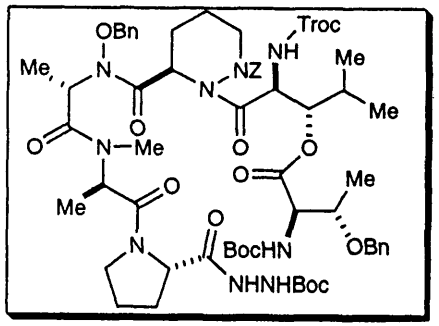

File:01SE4552 Ident:2 Acq: 3-DEC-2001 16:12:50 +0:18 Cal:FABMM031201_1
ZAB-SE4F FAB+ Magnet BpI:12609720 TIC:334427936 Flags:HALL
File Text:III-LL-21 FABMS MATRIX MNOBA + NA
OBn
Me
N
NZ
N
H
O
O
N—Me
Me
O
N
O
NHNHBoc
100% 219
263
241
334
356
401
288
316
424
437
509
552
604
638
652
738
760
777
830
870
m/z

PERKIN ELMER

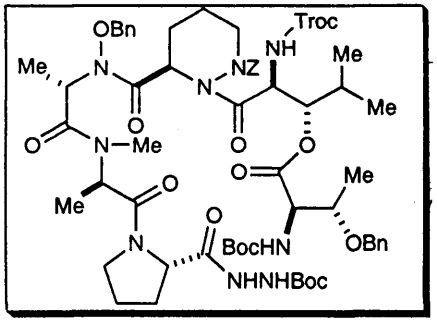


02/03/21 14:26
X: 16 scans, 4.0cm-1

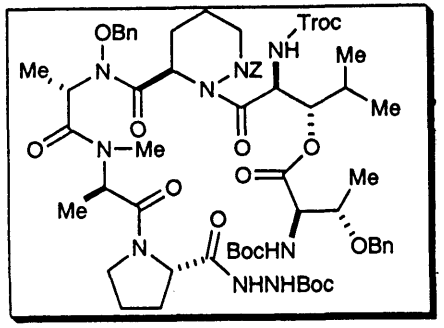
III-LL-24
In CDCl3 298K



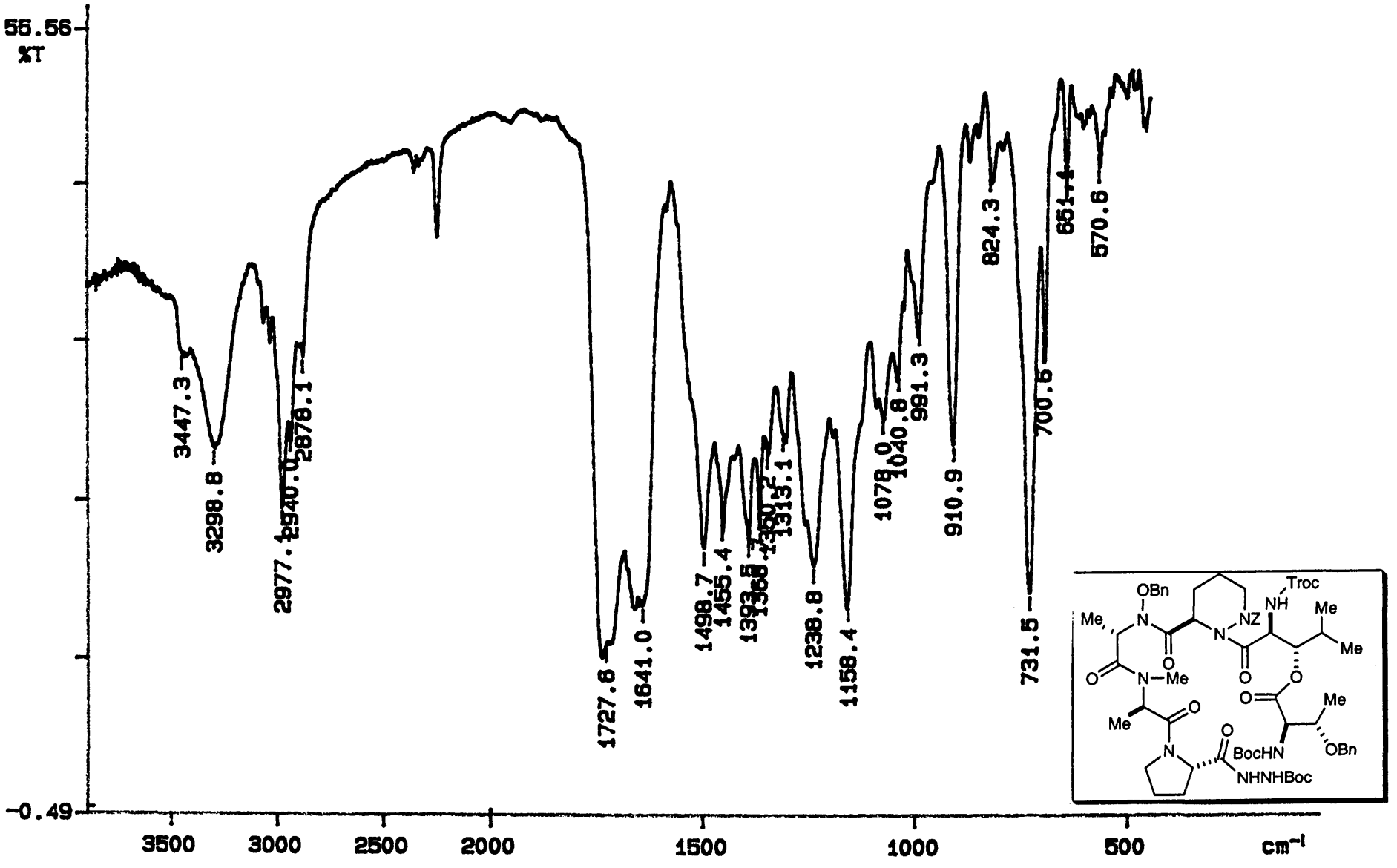
III-LL-24
In CDCl3 298K



File:01SE4551 Ident:8_12 Win 1000PPM Acq: 3-DEC-2001 15:45:28 +1:04 Cal:FABMM031201_1
ZAB-SE4F FAB+ Magnet BpM:133 BpI:44210588 TIC:301910176 Flags:HALL
File Text:III-LL-24 FABMS MATRIX MNOBA + NA

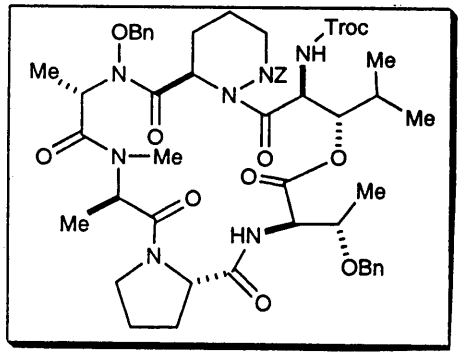


PERKIN ELMER

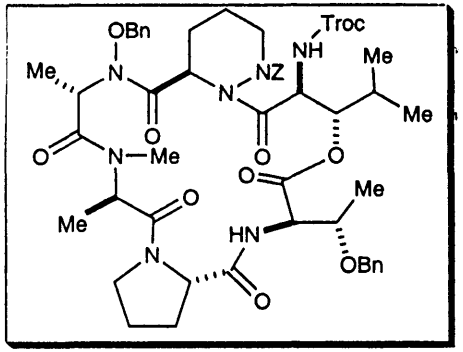


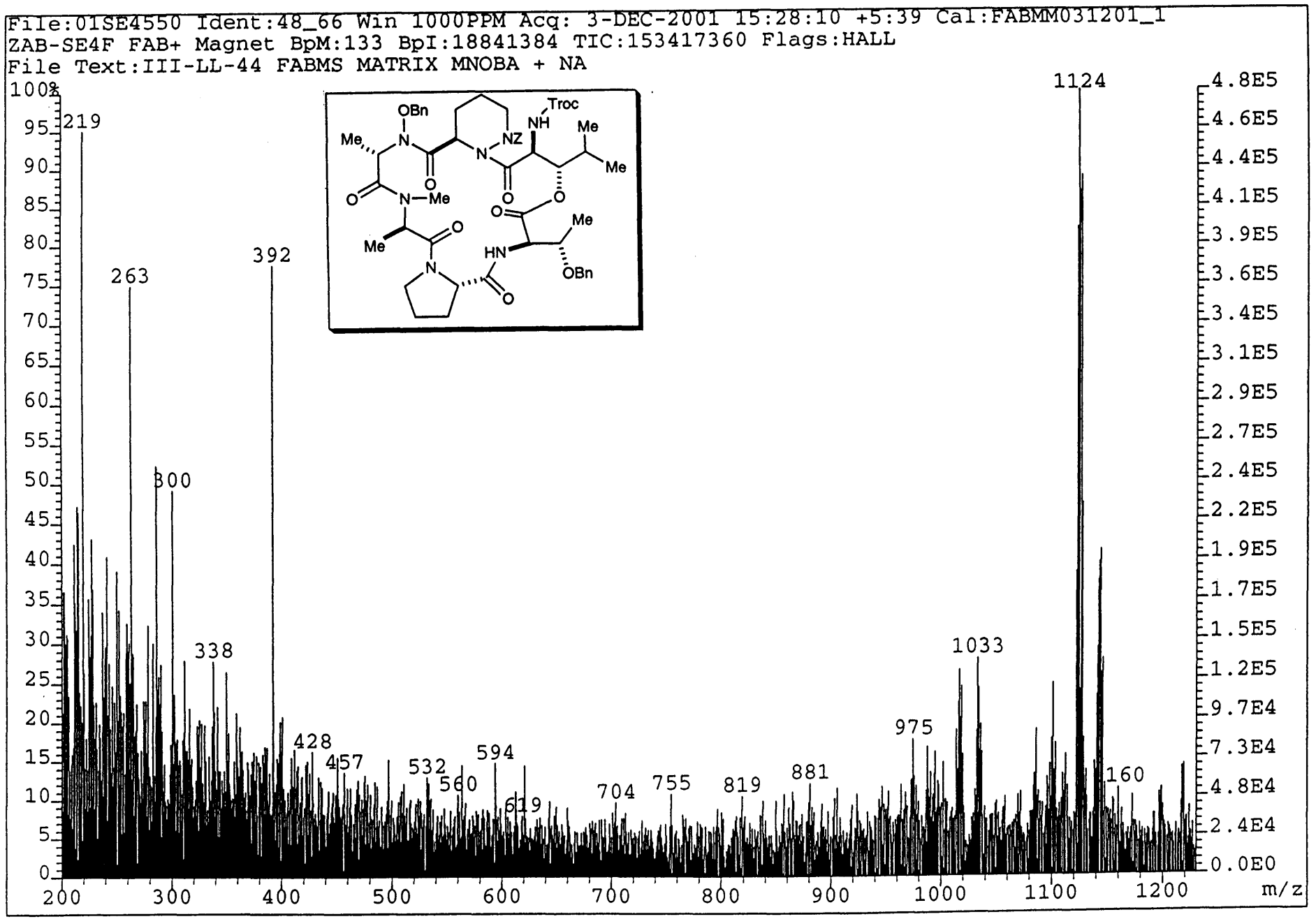
02/03/21 14:37
X: 16 scans, 4.0cm-1

III-LL-44
In CDCl3 298K

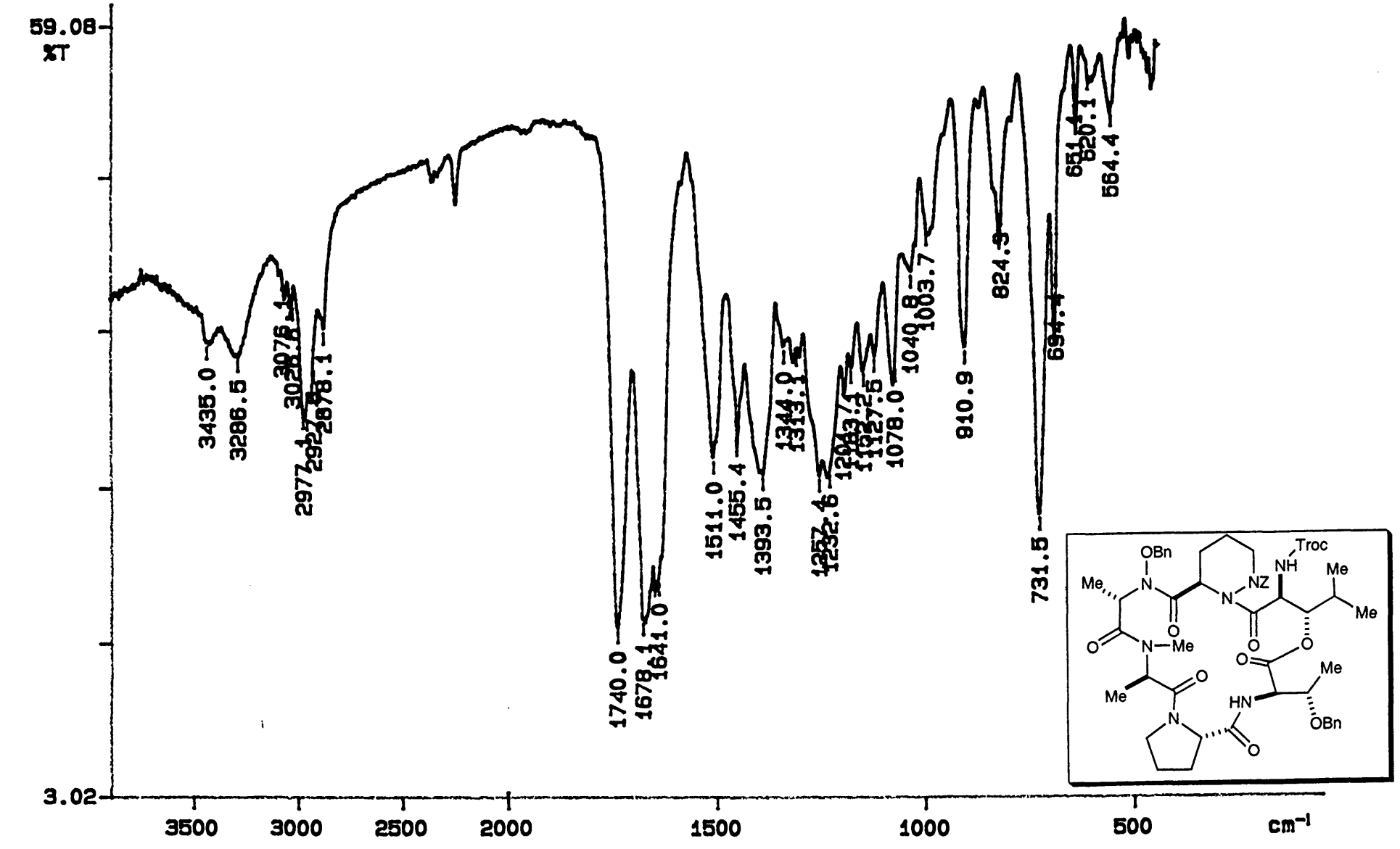


III-LL-44
In CDCl3 298K



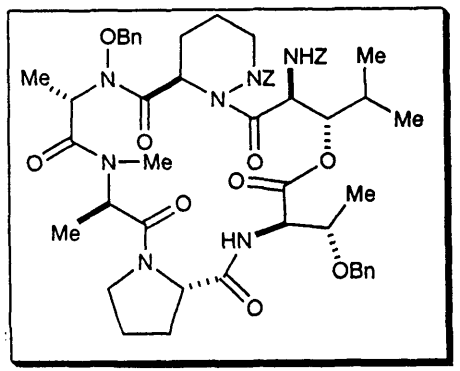

File:01SE4550 Ident:48_66 Win 1000PPM Acq: 3-DEC-2001 15:28:10 +5:39 Cal:FABMM031201_1
ZAB-SE4F FAB+ Magnet BpM:133 BpI:18841384 TIC:153417360 Flags:HALL
File Text:III-LL-44 FABMS MATRIX MNOBA + NA
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
4.8E5
4.6E5
4.4E5
4.1E5
3.9E5
3.6E5
3.4E5
3.1E5
2.9E5
2.7E5
2.4E5
2.2E5
1.9E5
1.7E5
1.5E5
1.2E5
9.7E4
7.3E4
4.8E4
2.4E4
0.0E0
200
300
400
500
600
700
800
900
1000
1100
1200
m/z
219
263
300
338
392
428
457
532
560
594
619
704
755
819
881
975
1033
1124
160
OBn
Troc
NH
Me
NZ
N
O
N–Me
HN
OBn

PERKIN ELMER

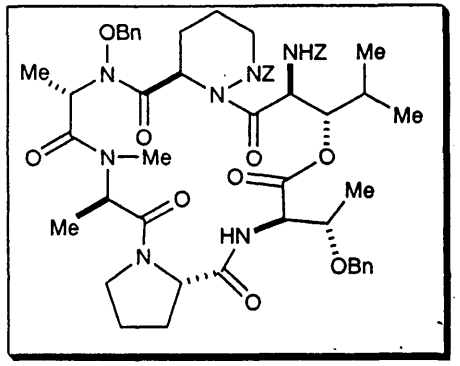


02/03/21 14: 49
X: 16 scans, 4.0cm-1

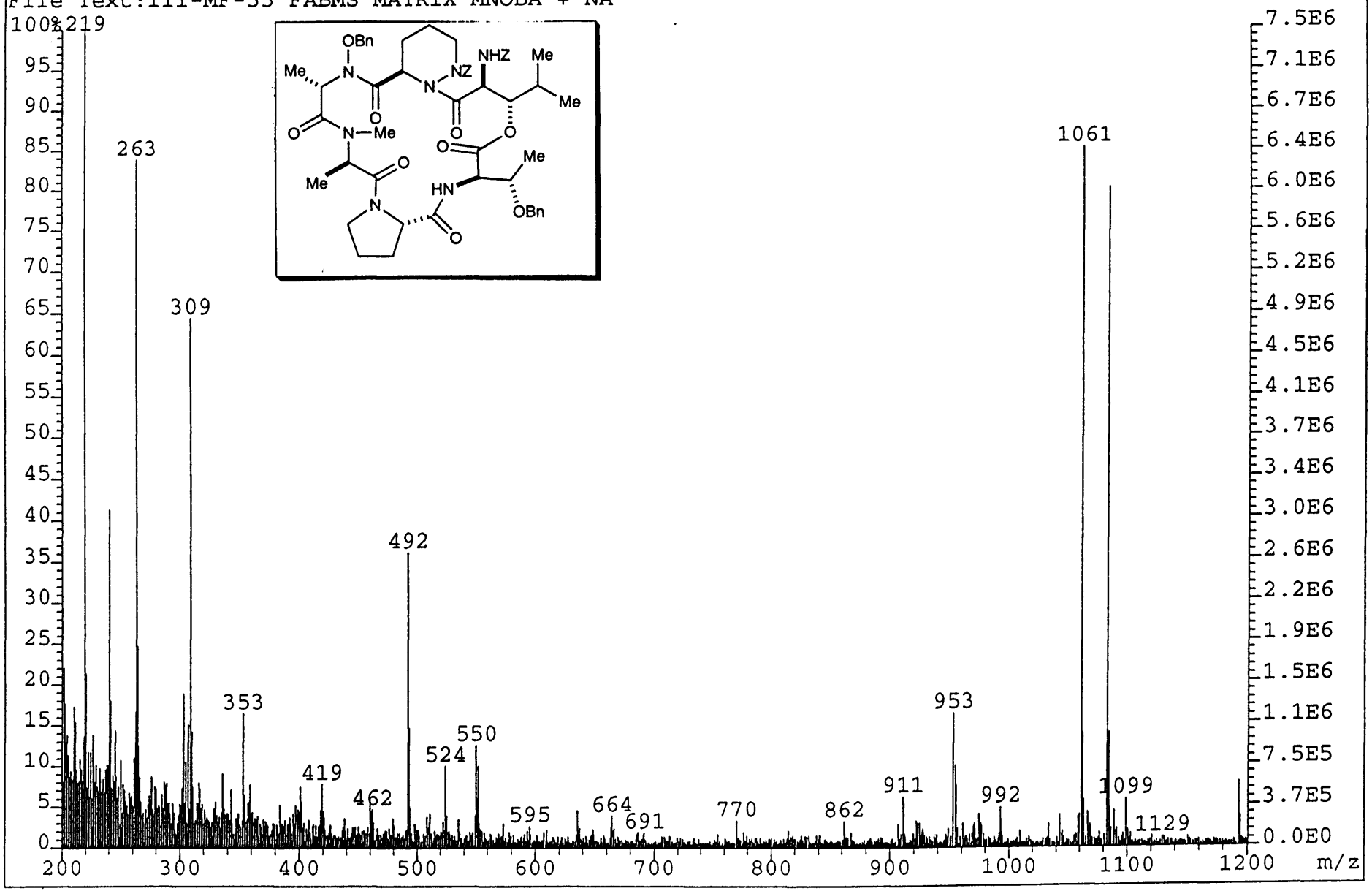
III-LL-55
In CDCl3 298K



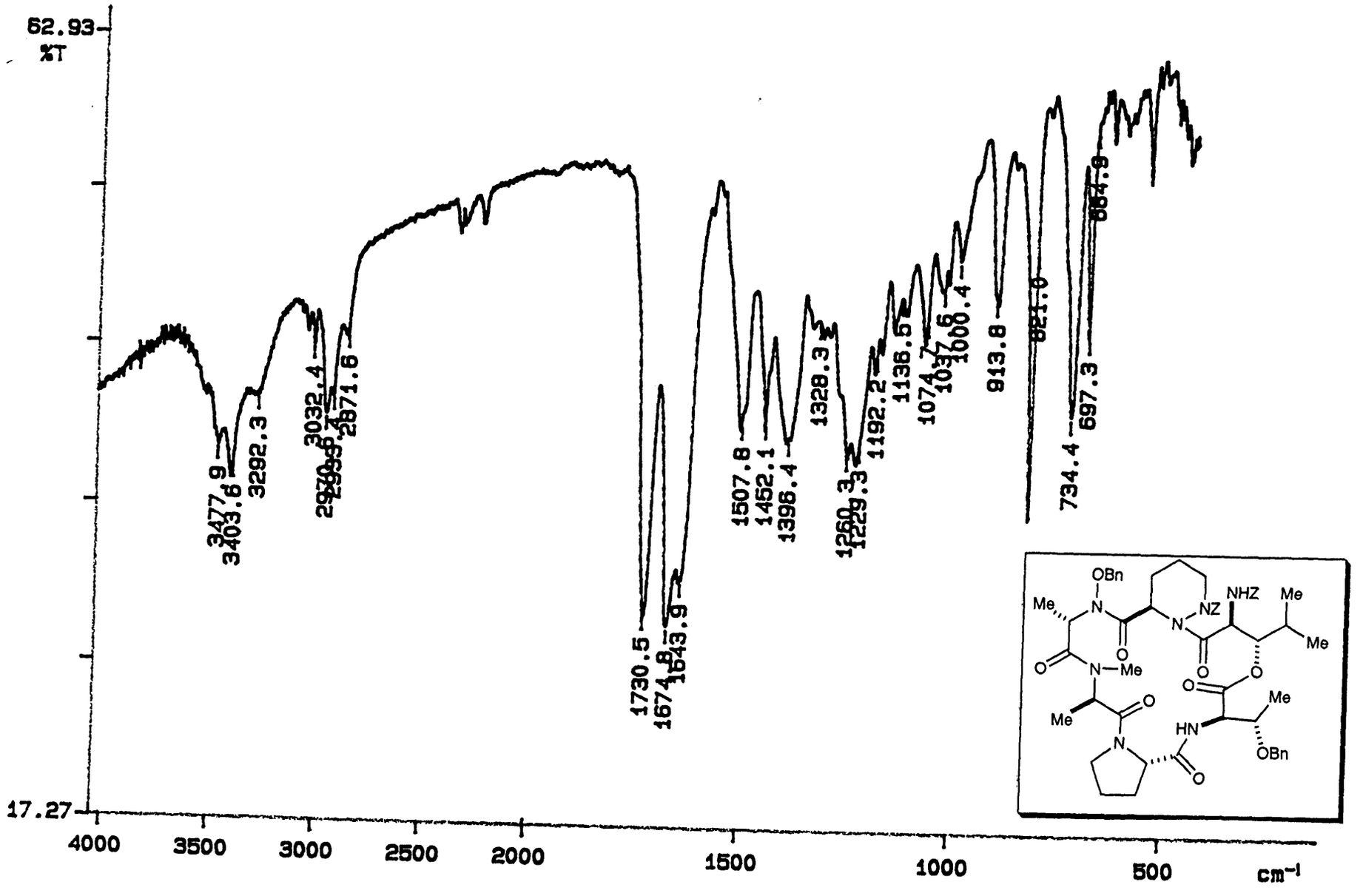
III-LL-55
In CDCl3 298K



File:01SE4563 Ident:4 Acq: 3-DEC-2001 16:44:41 +0:27 Cal:FABMM031201_1
ZAB-SE4F FAB+ Magnet BpI:10324246 TIC:531387648 Flags:HALL
File Text:III-MF-55 FABMS MATRIX MNOBA + NA

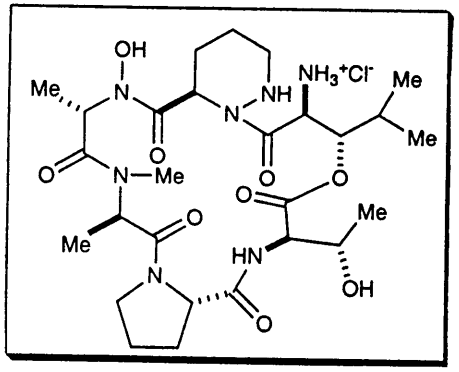


PERKIN ELMER

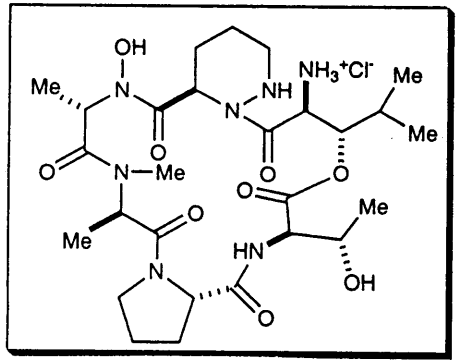


02/03/21 15: 01
X: 16 scans, 4.0cm-1

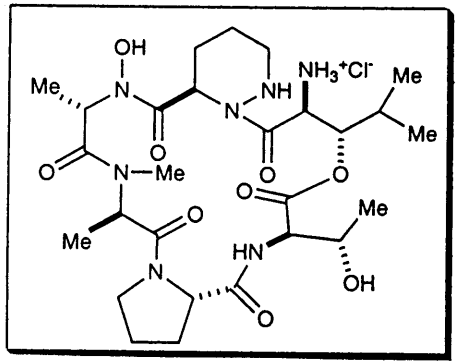
III-LL-56
In d-MeOH 298K



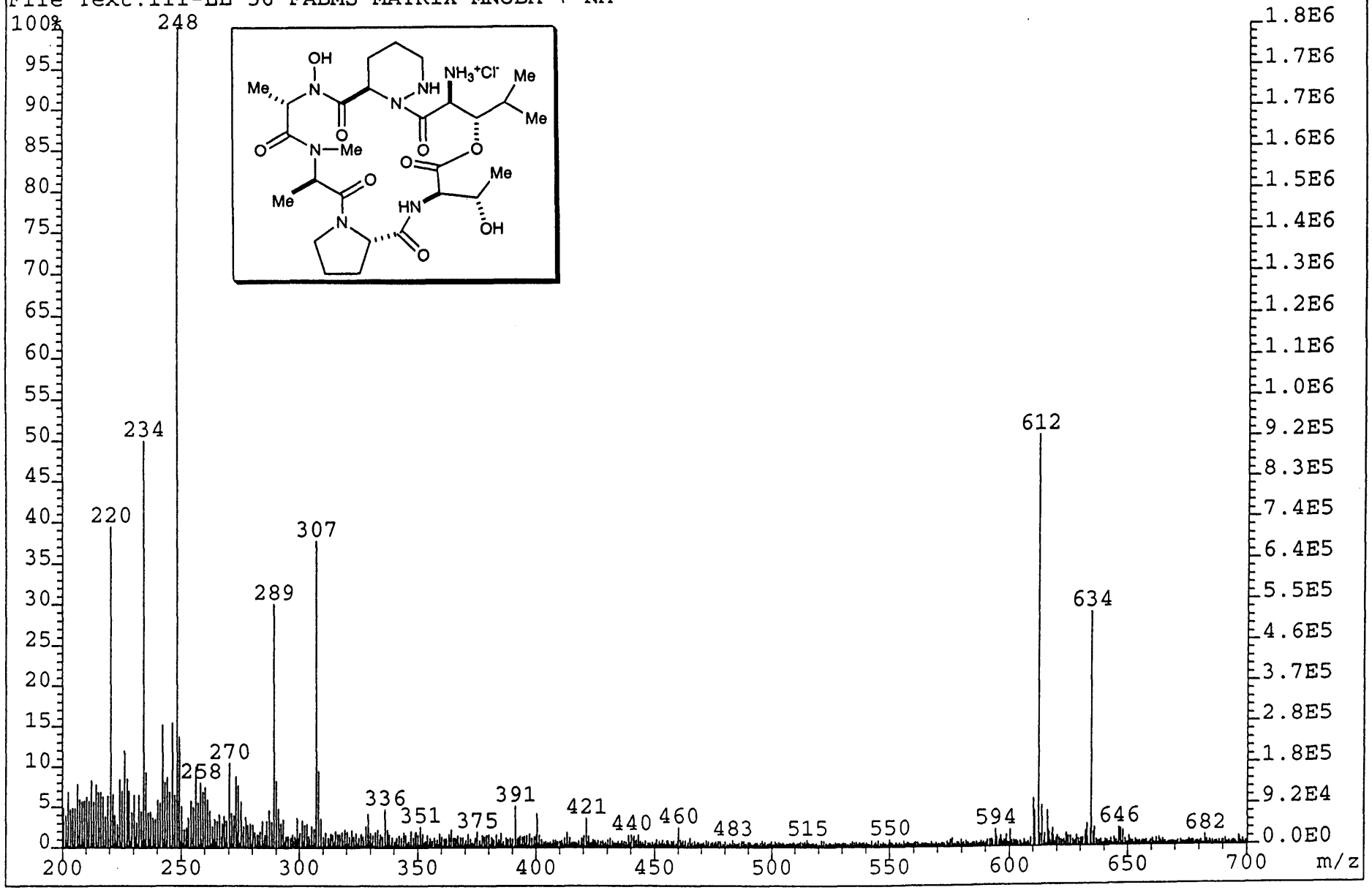
III-LL-56
In d-MeOH 298K

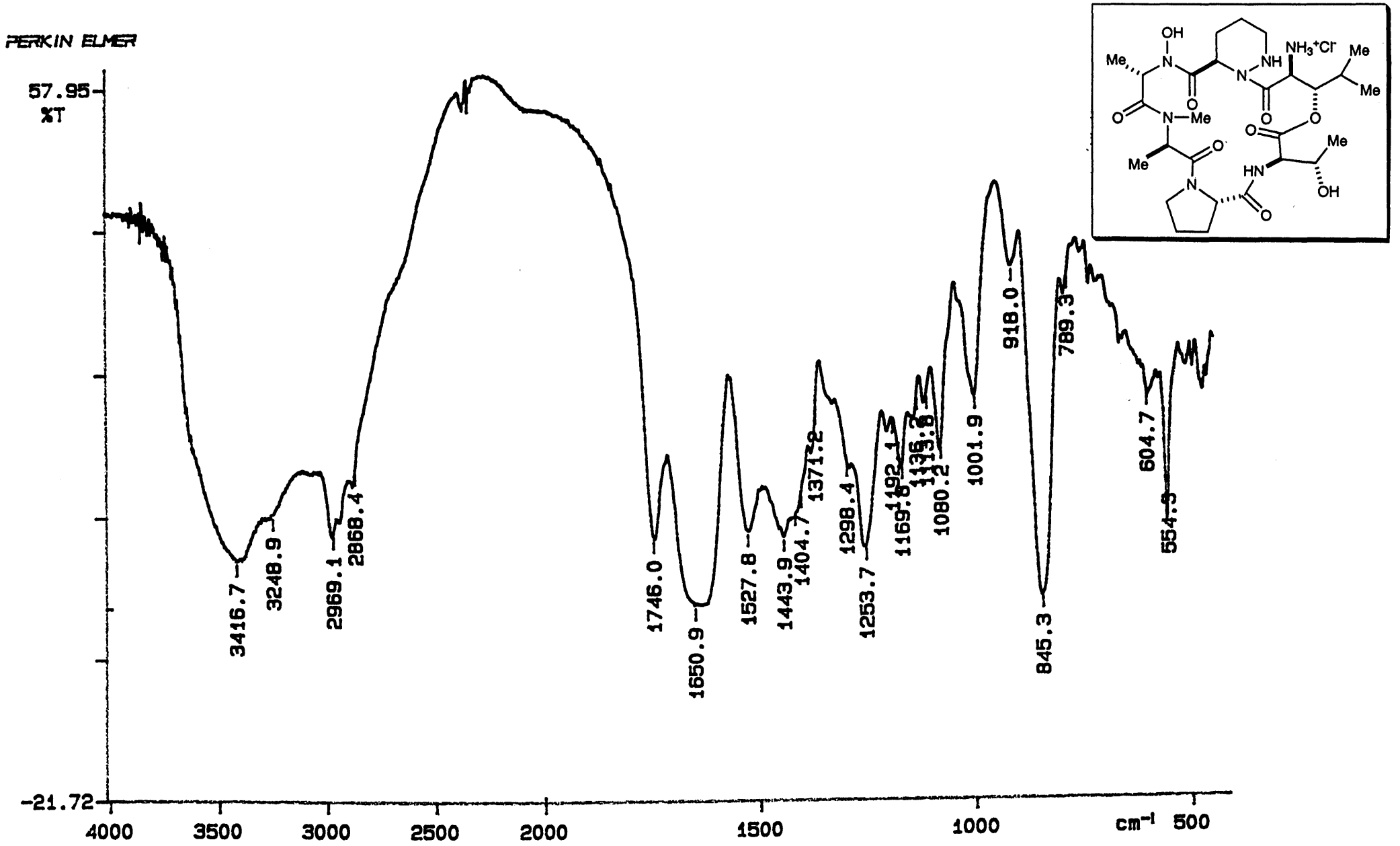


III-LL-56
In d-MeOH 298K

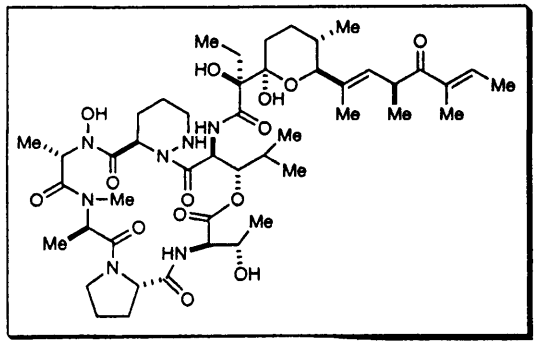


File:01SE4527 Ident:2_7 Win 1000PPM Acq:30-NOV-2001 11:02:54 +0:17 Cal:FABLM301101_1
ZAB-SE4F FAB+ Magnet BpM:136 BpI:8094379 TIC:110857384 Flags:HALL
File Text:III-LL-56 FABMS MATRIX MNOBA + NA

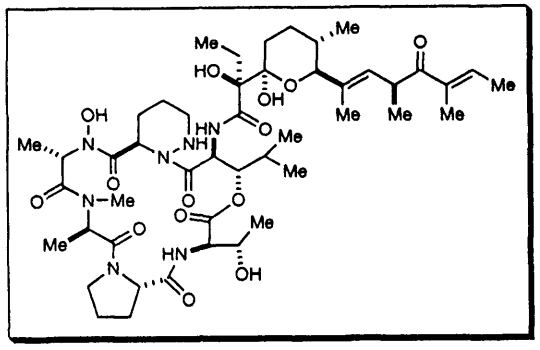



PERKIN ELMER
57.95
%T
-21.72
3416.7
3248.9
2969.1
2868.4
1746.0
1650.9
1527.8
1443.9
1404.7
1371.2
1298.4
1253.7
1192.1
1169.8
1136.6
1113.8
1080.2
1001.9
918.0
845.3
789.3
604.7
554.9
4000
3500
3000
2500
2000
1500
1000
cm-1
500
02/03/21 16:22
X: 16 scans, 4.0cm-1

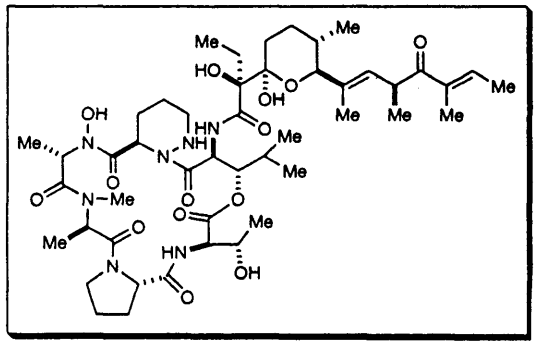
500MHz HNMR
298K In CDCl3



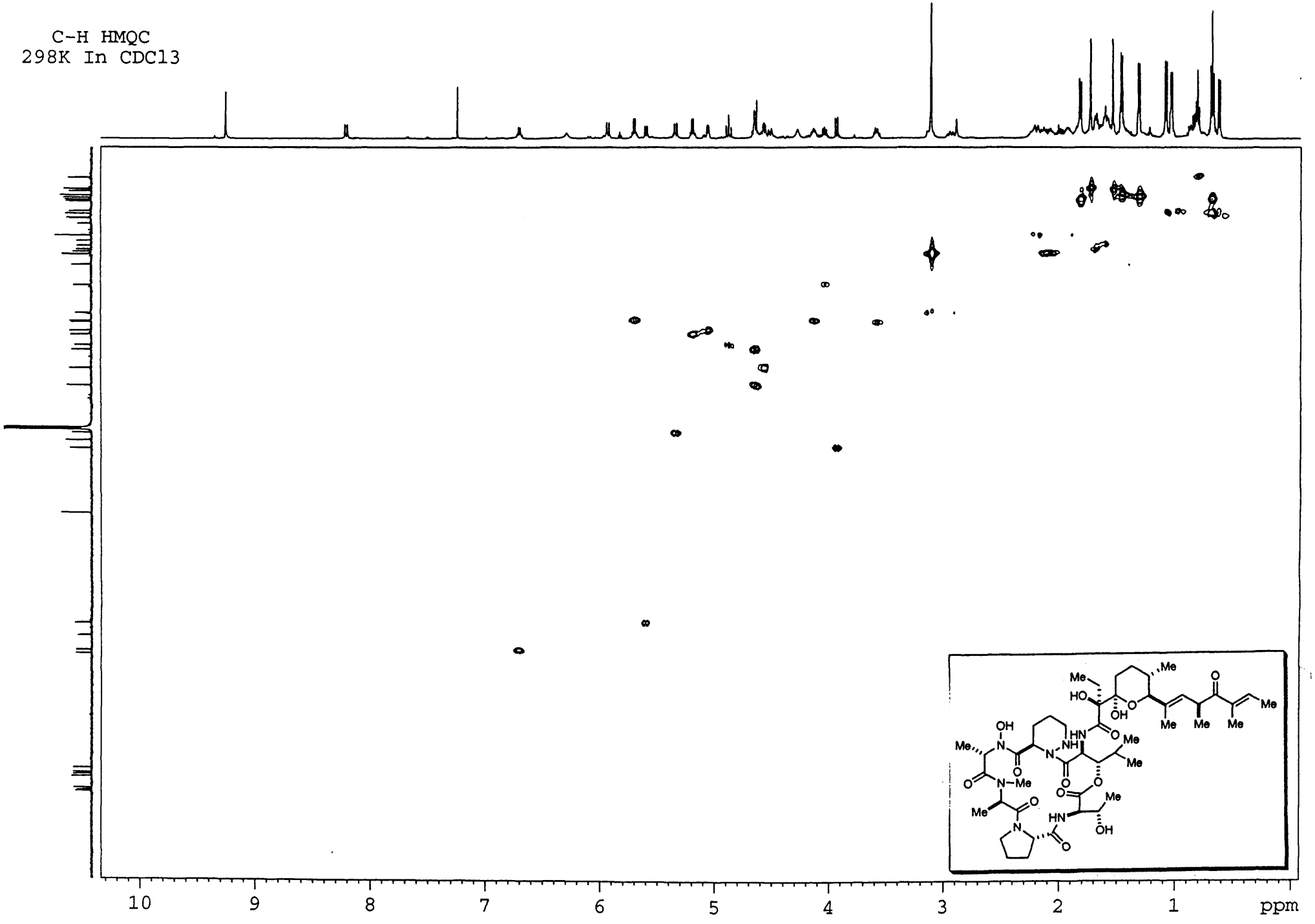
125MHz CNMR
298K In CDCl3



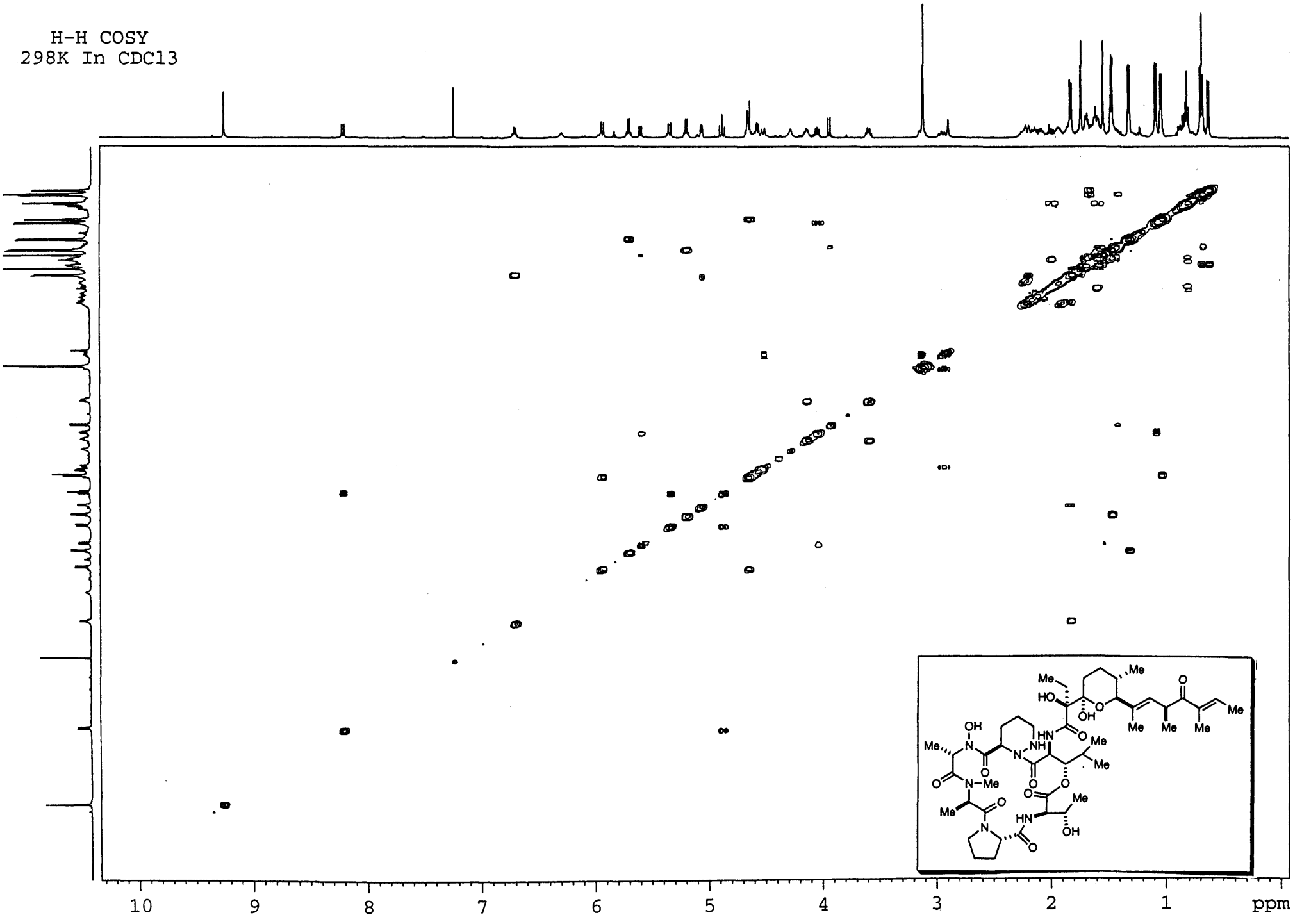
C DEPT
298K In CDCl3

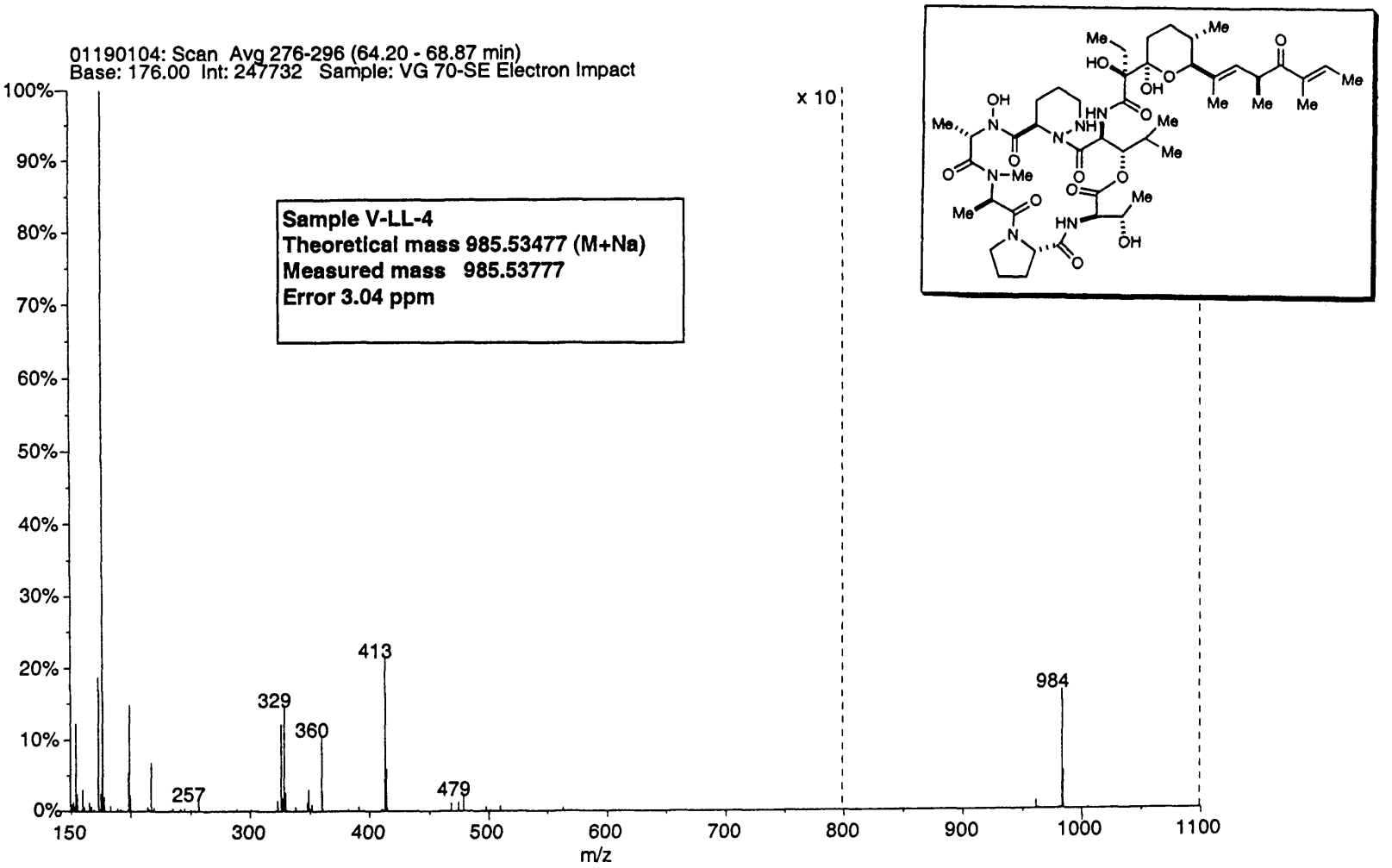


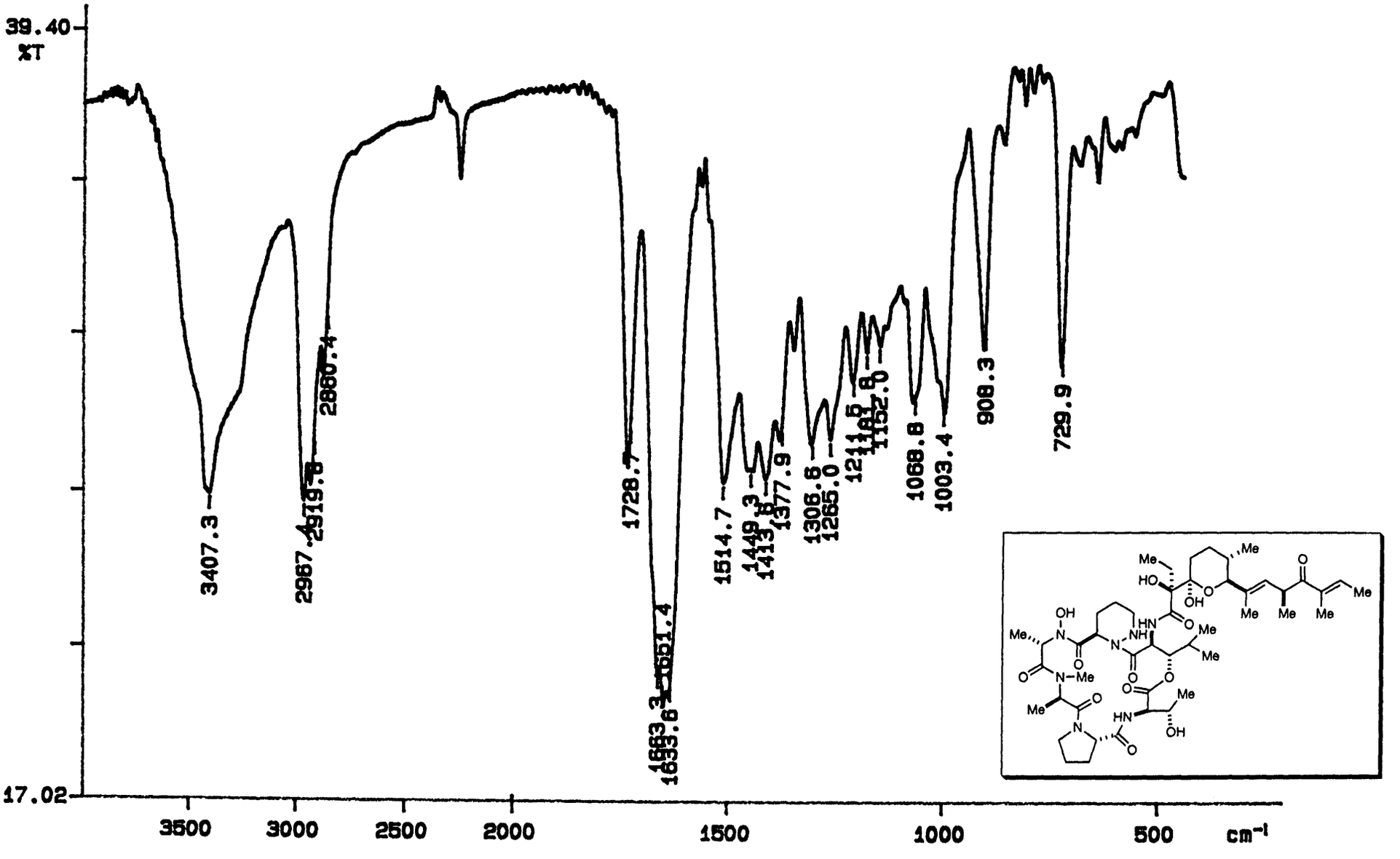
C-H HMQC
298K In CDCl3



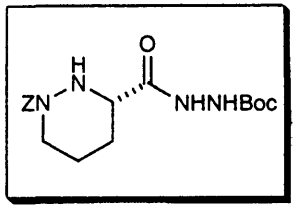
H-H COSY
298K In CDCl3



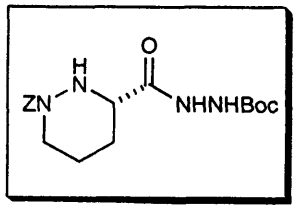

01190104: Scan Avg 276-296 (64.20 - 68.87 min)
Base: 176.00 Int: 247732 Sample: VG 70-SE Electron Impact
Sample V-LL-4
Theoretical mass 985.53477 (M+Na)
Measured mass 985.53777
Error 3.04 ppm
x 10
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
150
300
400
500
600
700
800
900
1000
1100
m/z
413
329
360
257
479
984


39.40
%T
17.02
3407.3
2967.4
2919.8
2880.4
1728.7
1663.3
1651.4
1514.7
1449.3
1413.8
1377.9
1306.8
1265.0
1211.5
1161.8
1152.0
1068.8
1003.4
908.3
729.9
3500
3000
2500
2000
1500
1000
500
cm⁻¹

III-LL-40

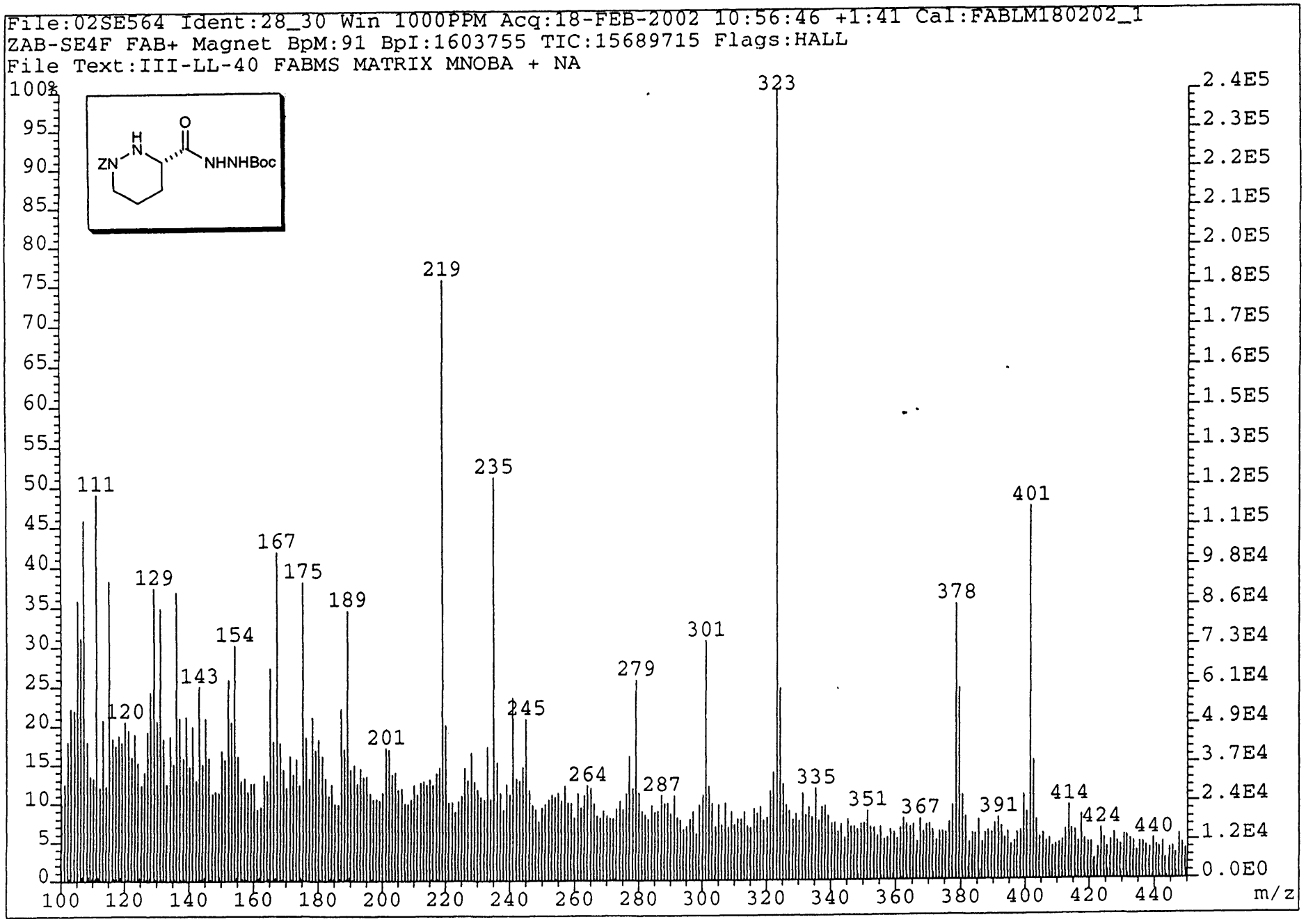


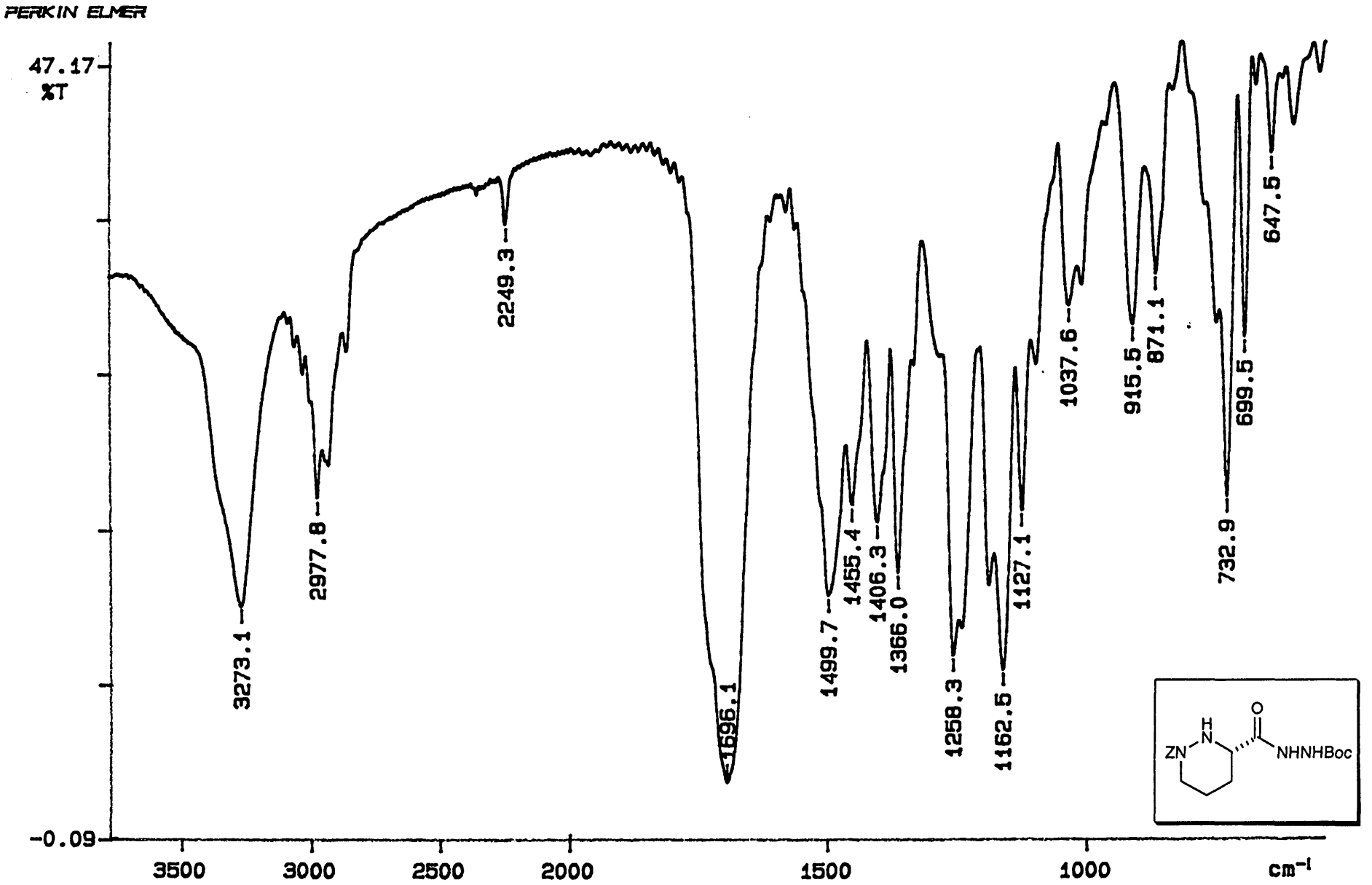
III-LL-40


O
H
N
ZN
NHNHBoc

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

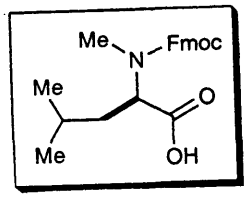
File:02SE564 Ident:28_30 Win 1000PPM Acq:18-FEB-2002 10:56:46 +1:41 Cal:FABLM180202_1
ZAB-SE4F FAB+ Magnet BpM:91 BpI:1603755 TIC:15689715 Flags:HALL
File Text:III-LL-40 FABMS MATRIX MNOBA + NA



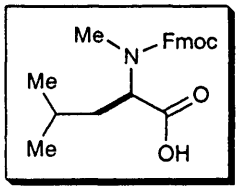


02/03/13 16:30
X: 16 scans, 16.0cm-1, apod none

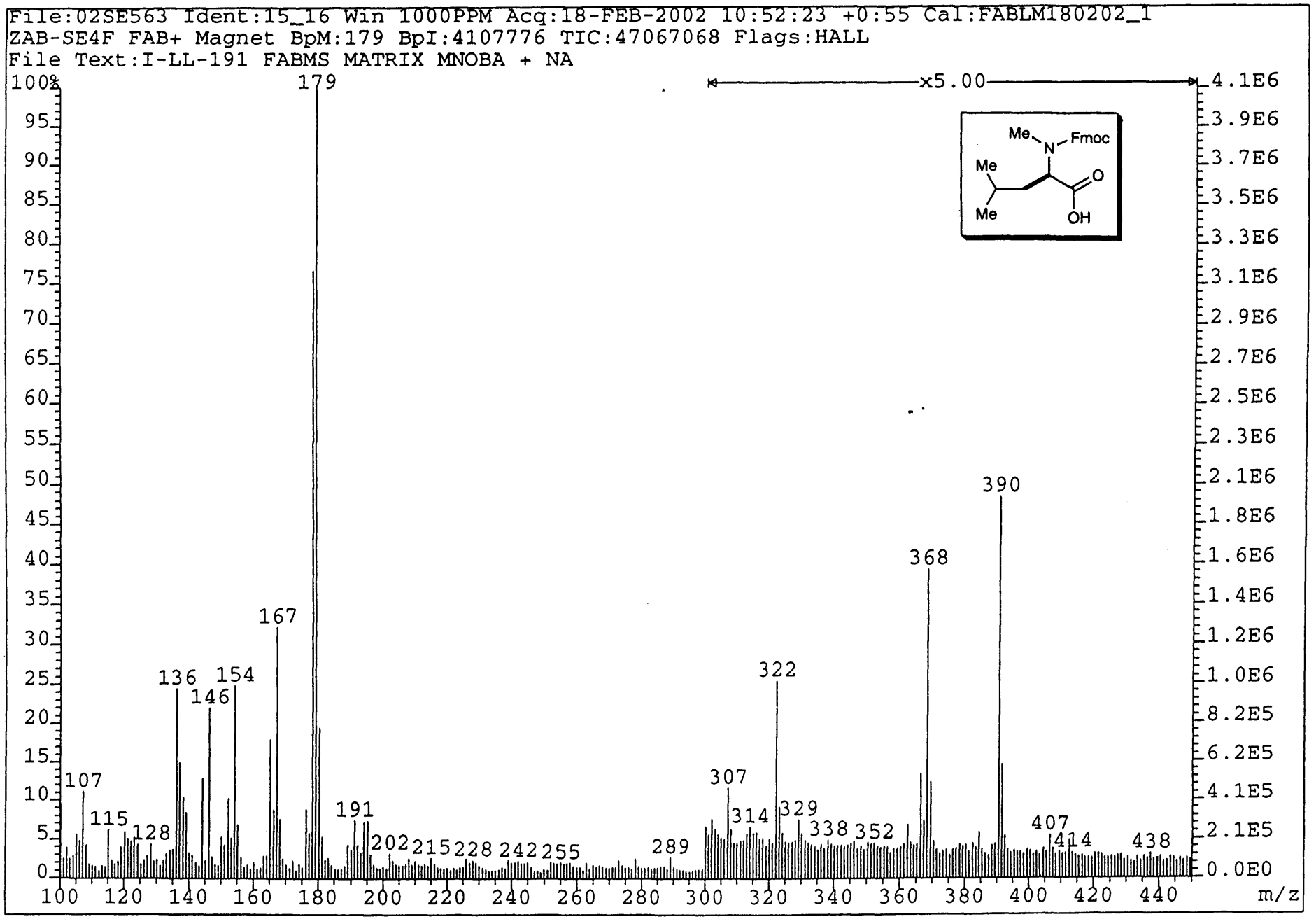
I-LL-191



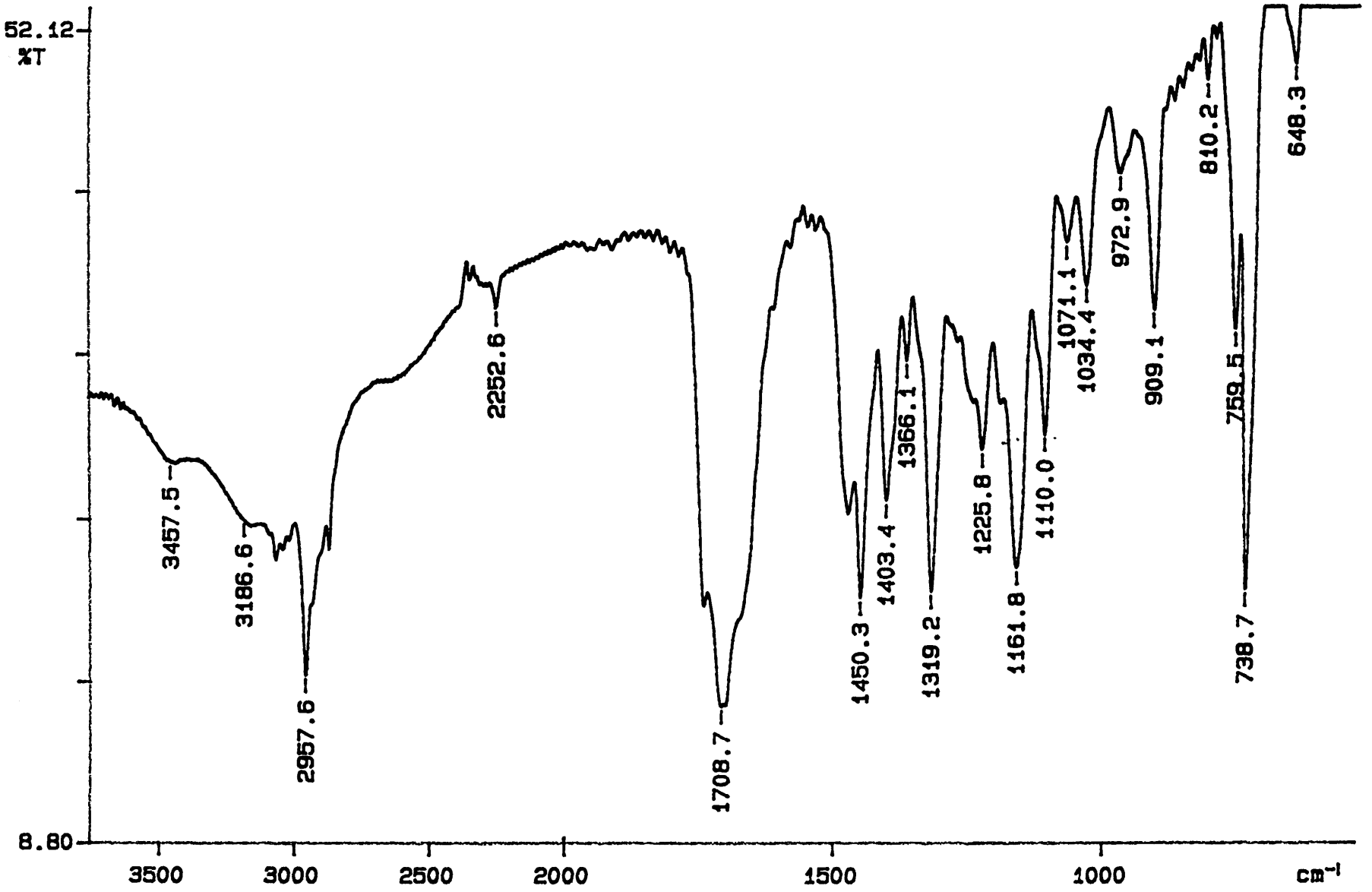
I-LL-191

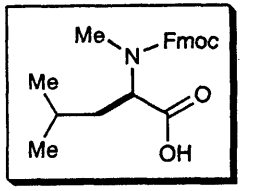

Me
N
Fmoc
Me
O
Me
OH

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


File:02SE563 Ident:15_16 Win 1000PPM Acq:18-FEB-2002 10:52:23 +0:55 Cal:FABLM180202_1
ZAB-SE4F FAB+ Magnet BpM:179 BpI:4107776 TIC:47067068 Flags:HALL
File Text:I-LL-191 FABMS MATRIX MNOBA + NA
x5.00
Me
N
Fmoc
Me
O
Me
OH
179
167
136
154
146
107
115
128
191
202
215
228
242
255
289
307
314
322
329
338
352
368
390
407
414
438
m/z

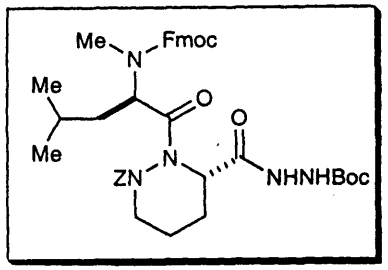
PERKIN ELMER



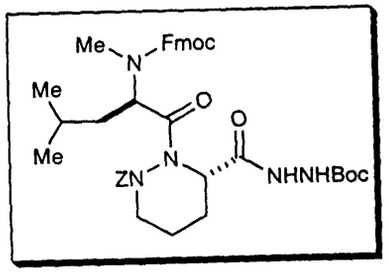


02/03/13 16: 44
X: 16 scans, 16.0cm-1, apod none

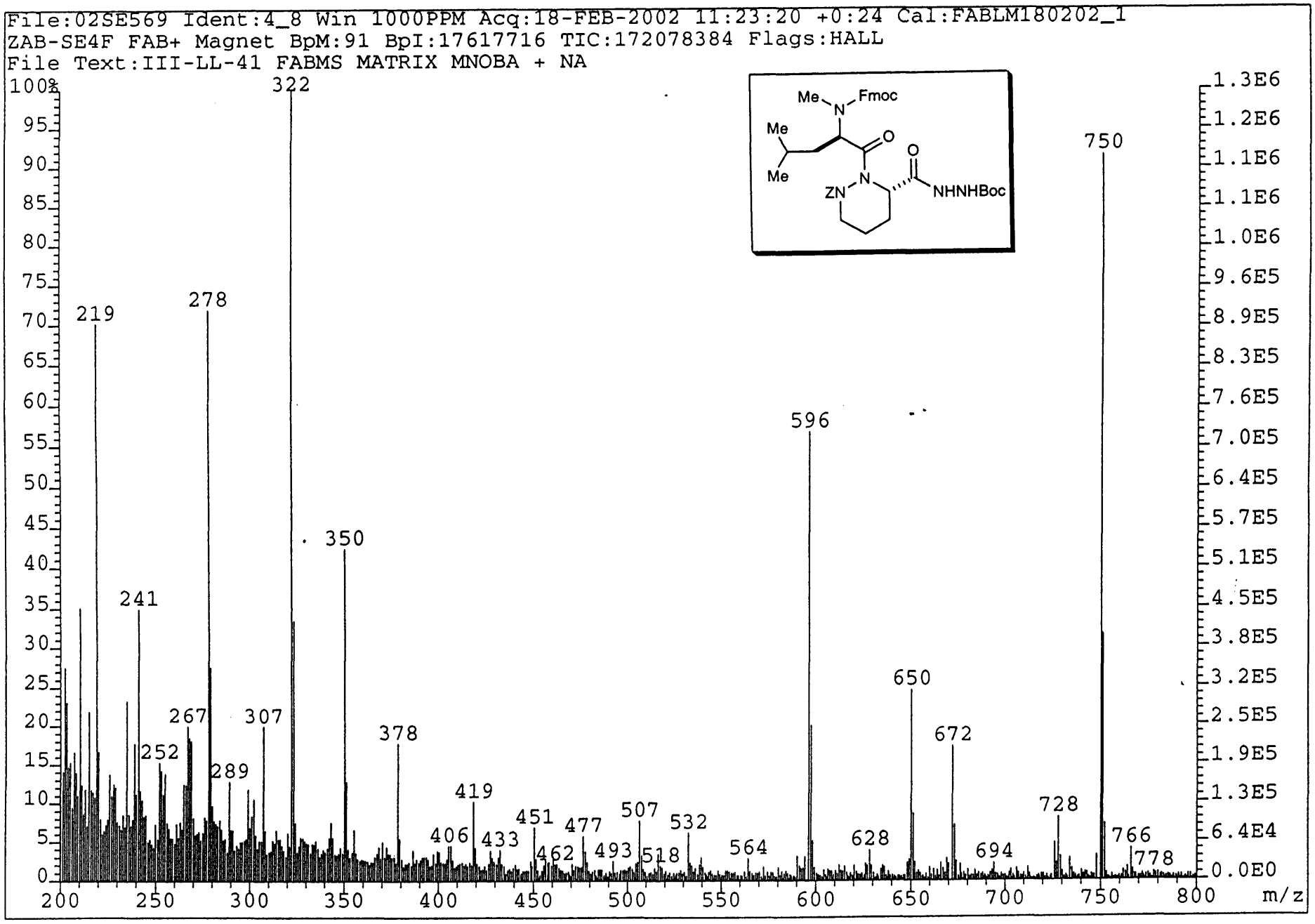
III-LL-41



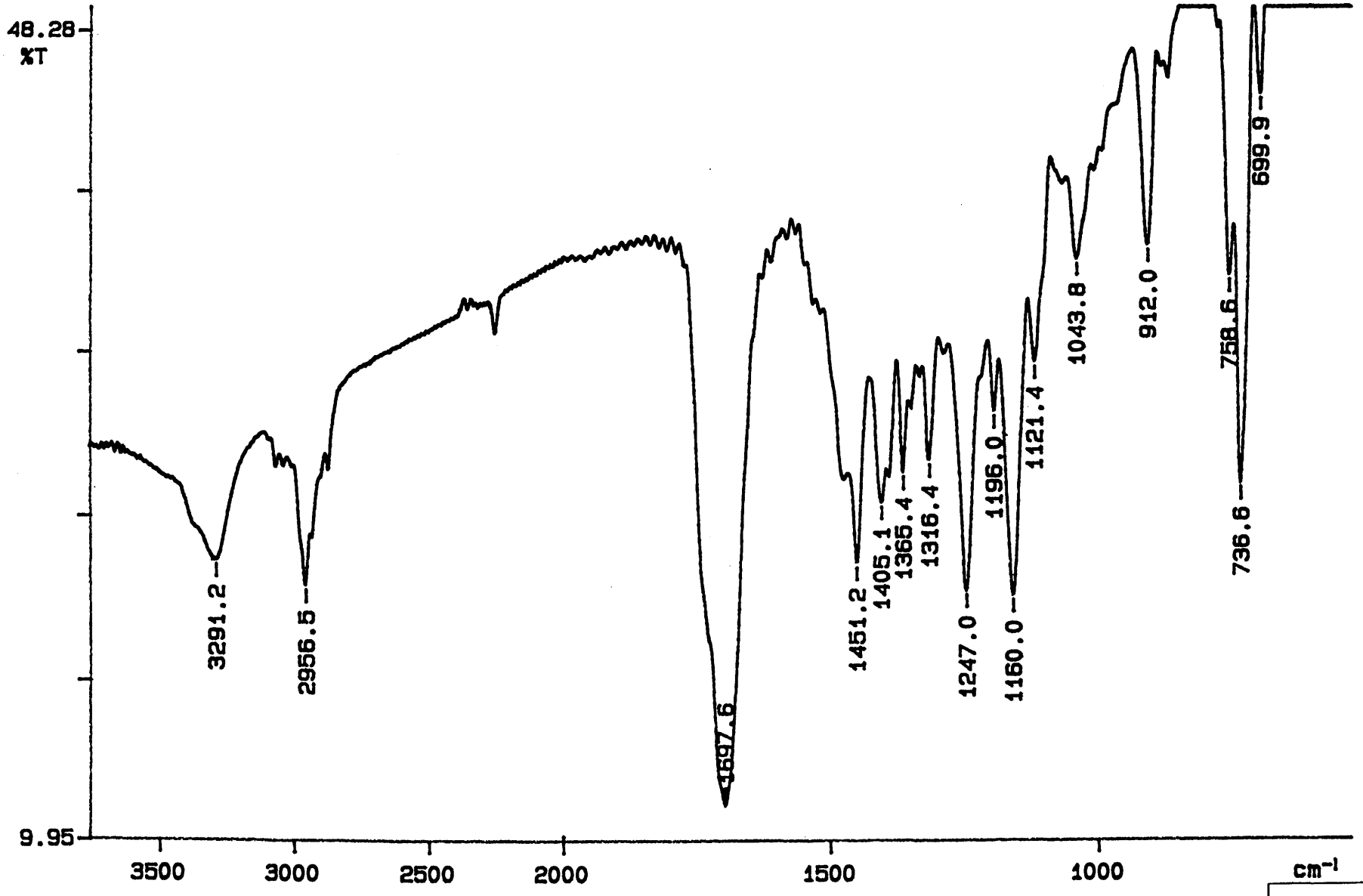
III-LL-41

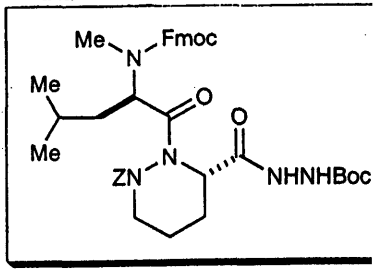

Me
N
Fmoc
Me
O
O
Me
N
ZN
NHNHBoc

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


File:02SE569 Ident:4_8 Win 1000PPM Acq:18-FEB-2002 11:23:20 +0:24 Cal:FABLM180202_1
ZAB-SE4F FAB+ Magnet BpM:91 BpI:17617716 TIC:172078384 Flags:HALL
File Text:III-LL-41 FABMS MATRIX MNOBA + NA
Me
N
Fmoc
Me
Me
O
O
ZN
N
NHNHBoc
322
750
596
278
219
350
241
650
672
267
307
378
252
289
419
728
451
477
507
532
406
433
462
493
518
564
628
694
766
778
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
1.3E6
1.2E6
1.1E6
1.1E6
1.0E6
9.6E5
8.9E5
8.3E5
7.6E5
7.0E5
6.4E5
5.7E5
5.1E5
4.5E5
3.8E5
3.2E5
2.5E5
1.9E5
1.3E5
6.4E4
0.0E0
200
250
300
350
400
450
500
550
600
650
700
750
800
m/z

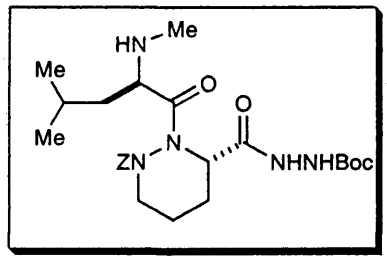
PERKIN ELMER



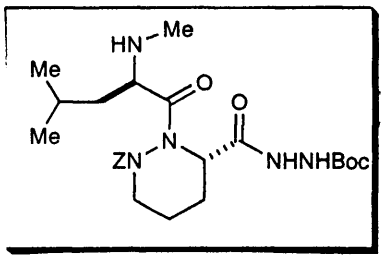


02/03/13 17:09
X: 16 scans, 16.0cm-1, apod none

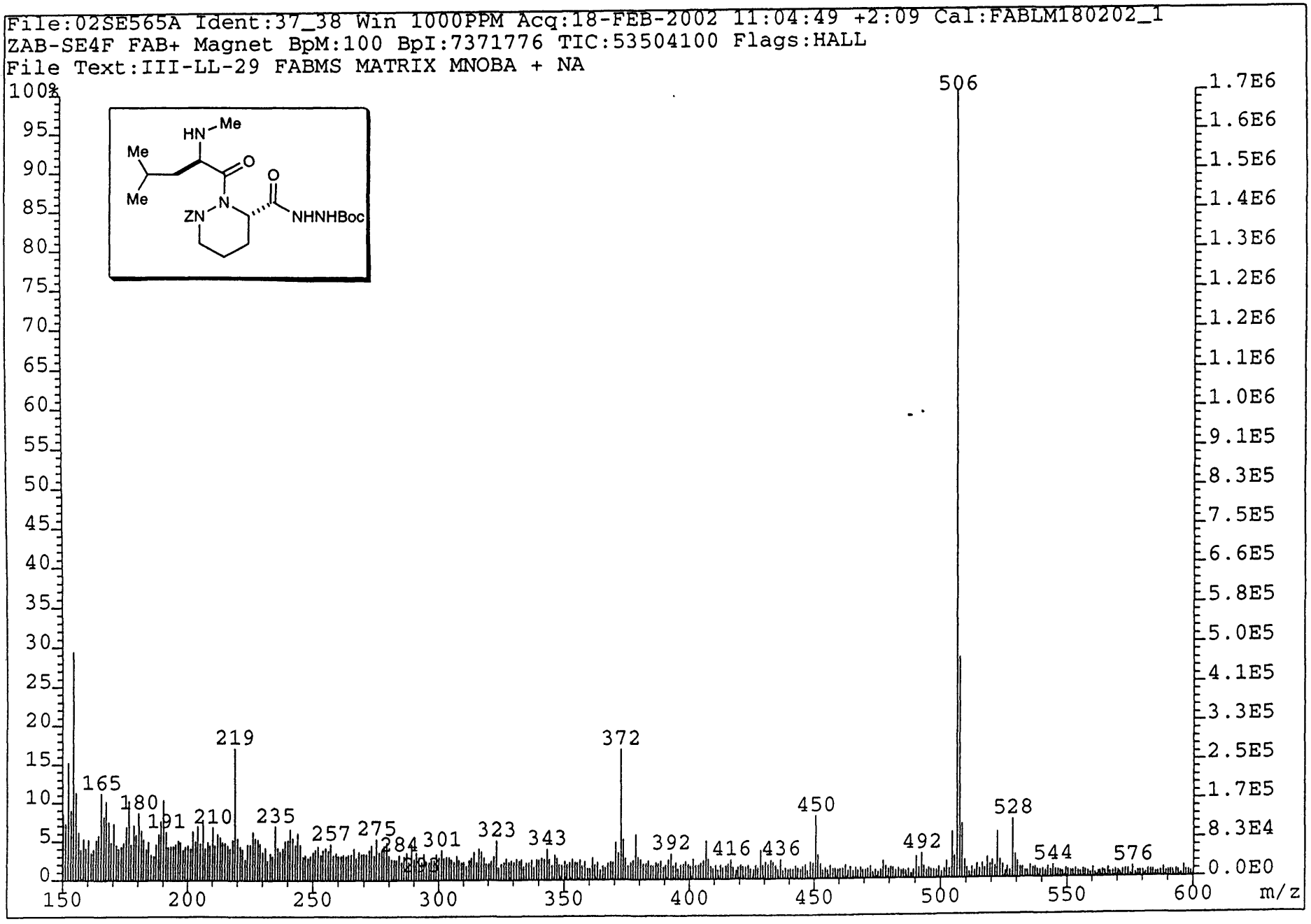
III-LL-29



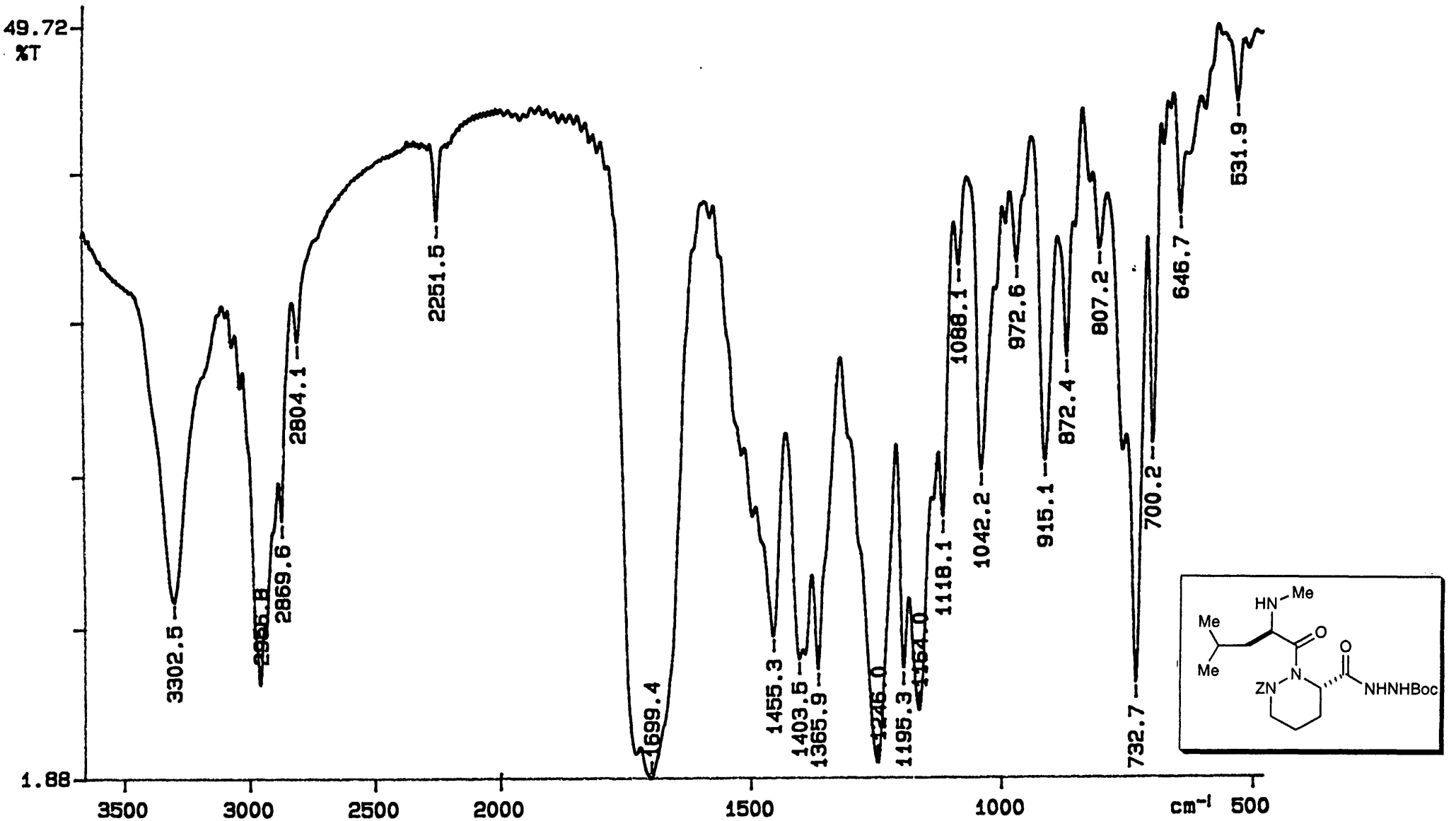
III-LL-29


HN
Me
Me
Me
O
O
N
ZN
NHNHBoc

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

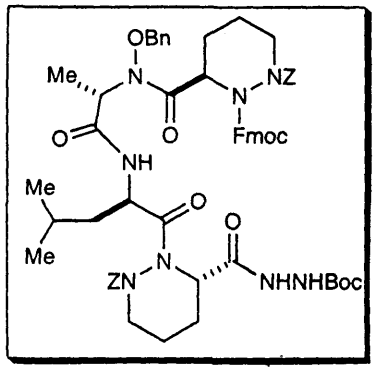

File:02SE565A Ident:37_38 Win 1000PPM Acq:18-FEB-2002 11:04:49 +2:09 Cal:FABLM180202_1
ZAB-SE4F FAB+ Magnet BpM:100 BpI:7371776 TIC:53504100 Flags:HALL
File Text:III-LL-29 FABMS MATRIX MNOBA + NA
HN Me
Me
Me
O
O
N
ZN
NHNHBoc
506
219
372
165
180
191
210
235
257
275
284
301
323
343
392
416
436
450
492
528
544
576
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
1.7E6
1.6E6
1.5E6
1.4E6
1.3E6
1.2E6
1.2E6
1.1E6
1.0E6
9.1E5
8.3E5
7.5E5
6.6E5
5.8E5
5.0E5
4.1E5
3.3E5
2.5E5
1.7E5
8.3E4
0.0E0
150
200
250
300
350
400
450
500
550
600
m/z

PERKIN ELMER

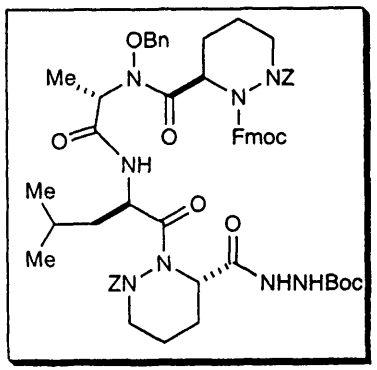


02/03/13 17:23
X: 16 scans, 16.0cm-1, apod none

III-LL-68

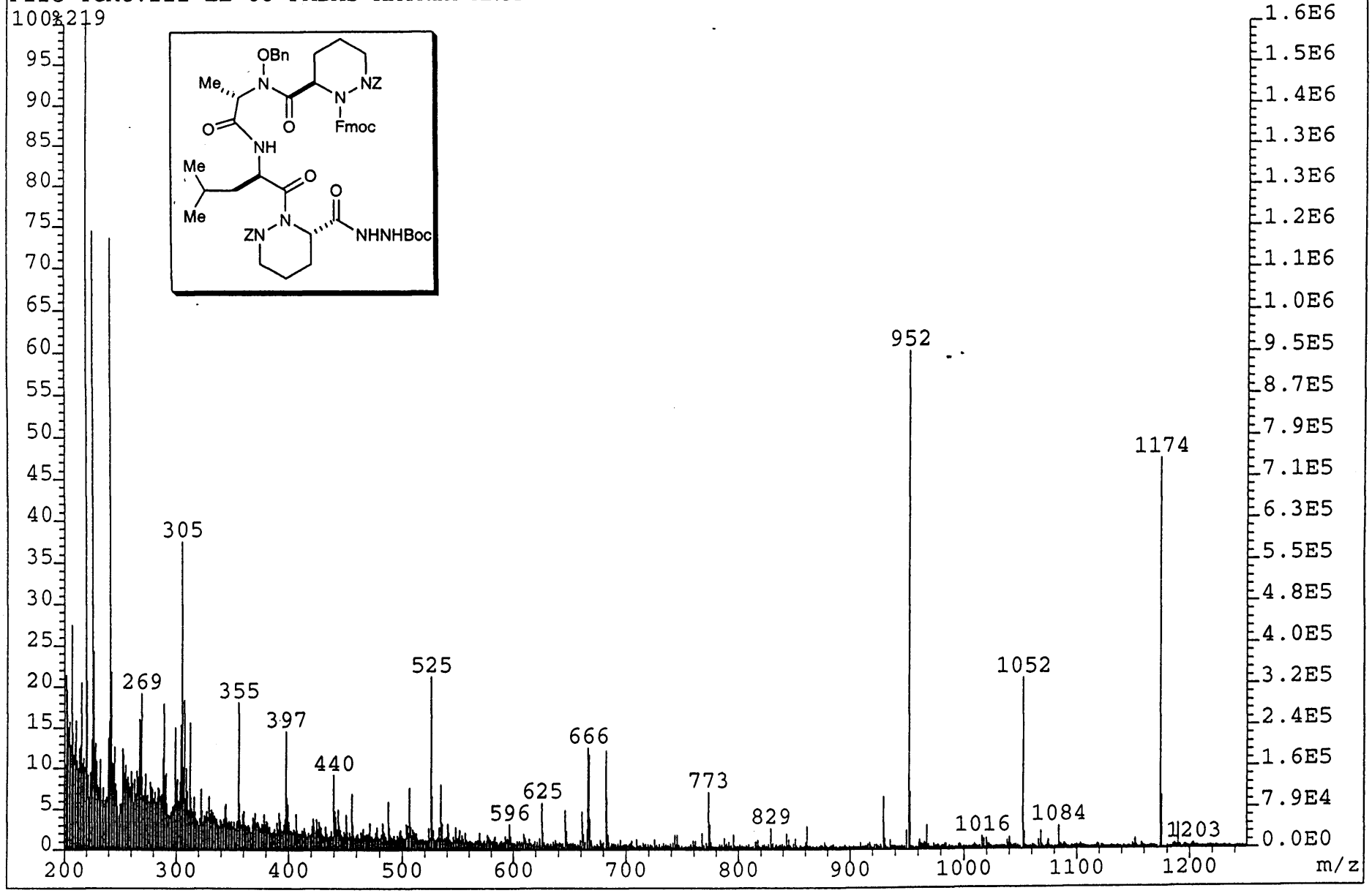


III-LL-68

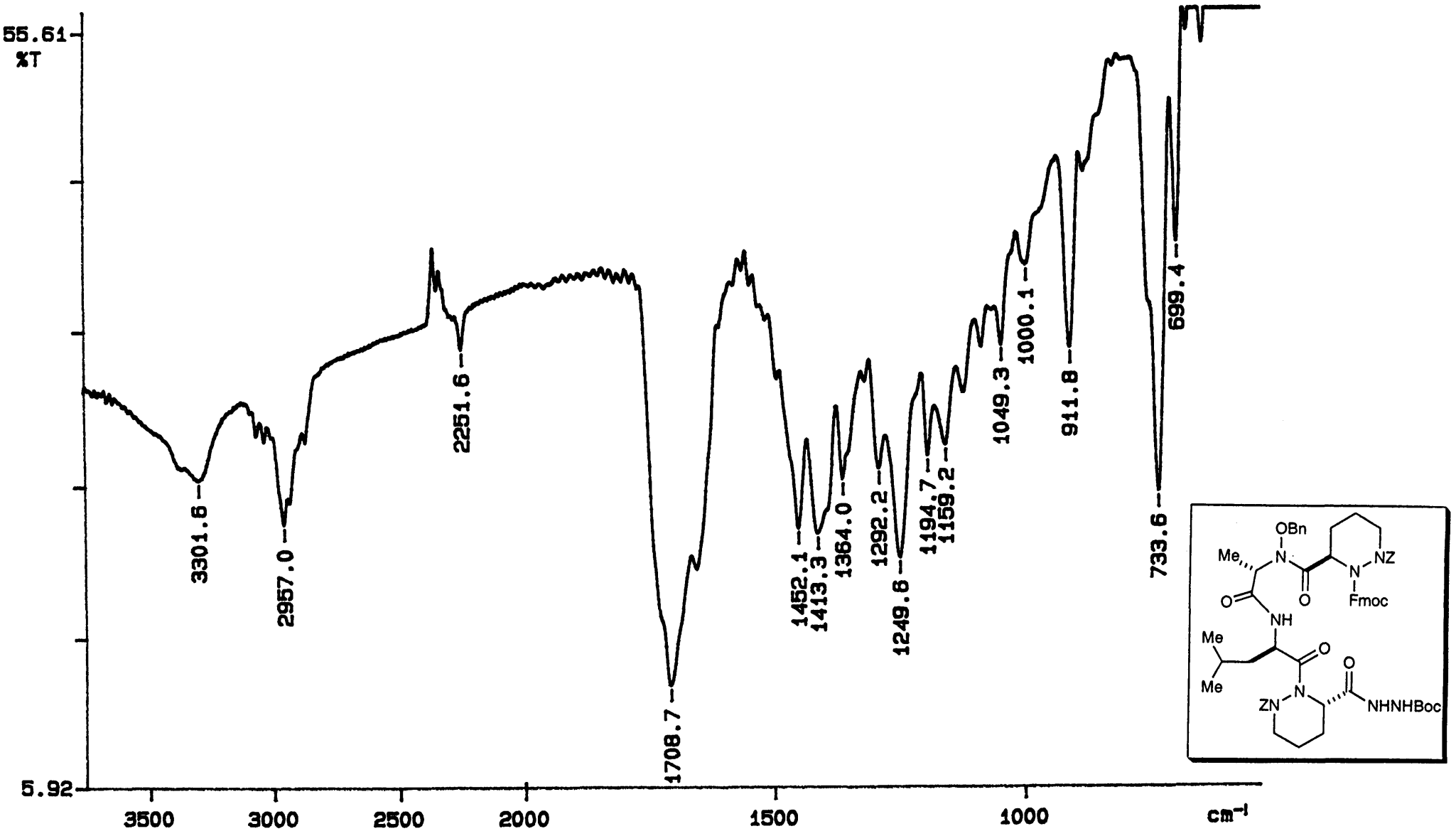

OBn
Me
N
NZ
O
N
O
Fmoc
NH
Me
O
Me
O
N
ZN
NHNHBoc

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

File:02SE572 Ident:15_37 Win 1000PPM Acq:18-FEB-2002 12:17:43 +1:31 Cal:FABLM180202_1
ZAB-SE4F FAB+ Magnet BpM:179 BpI:12165833 TIC:163061952 Flags:HALL
File Text:III-LL-68 FABMS MATRIX MNOBA + NA

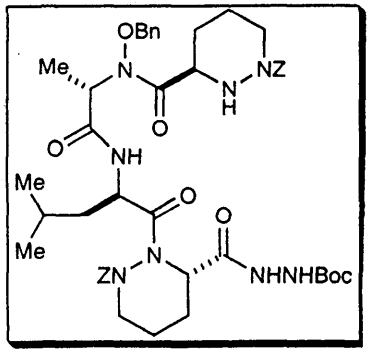


PERKIN ELMER

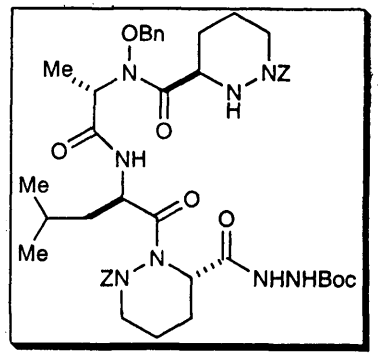


02/03/14 13:04
X: 16 scans, 16.0cm-1, apod none

III-LL-69

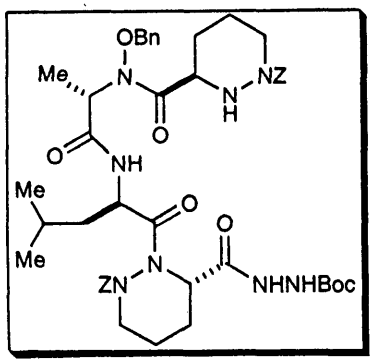


III-LL-69

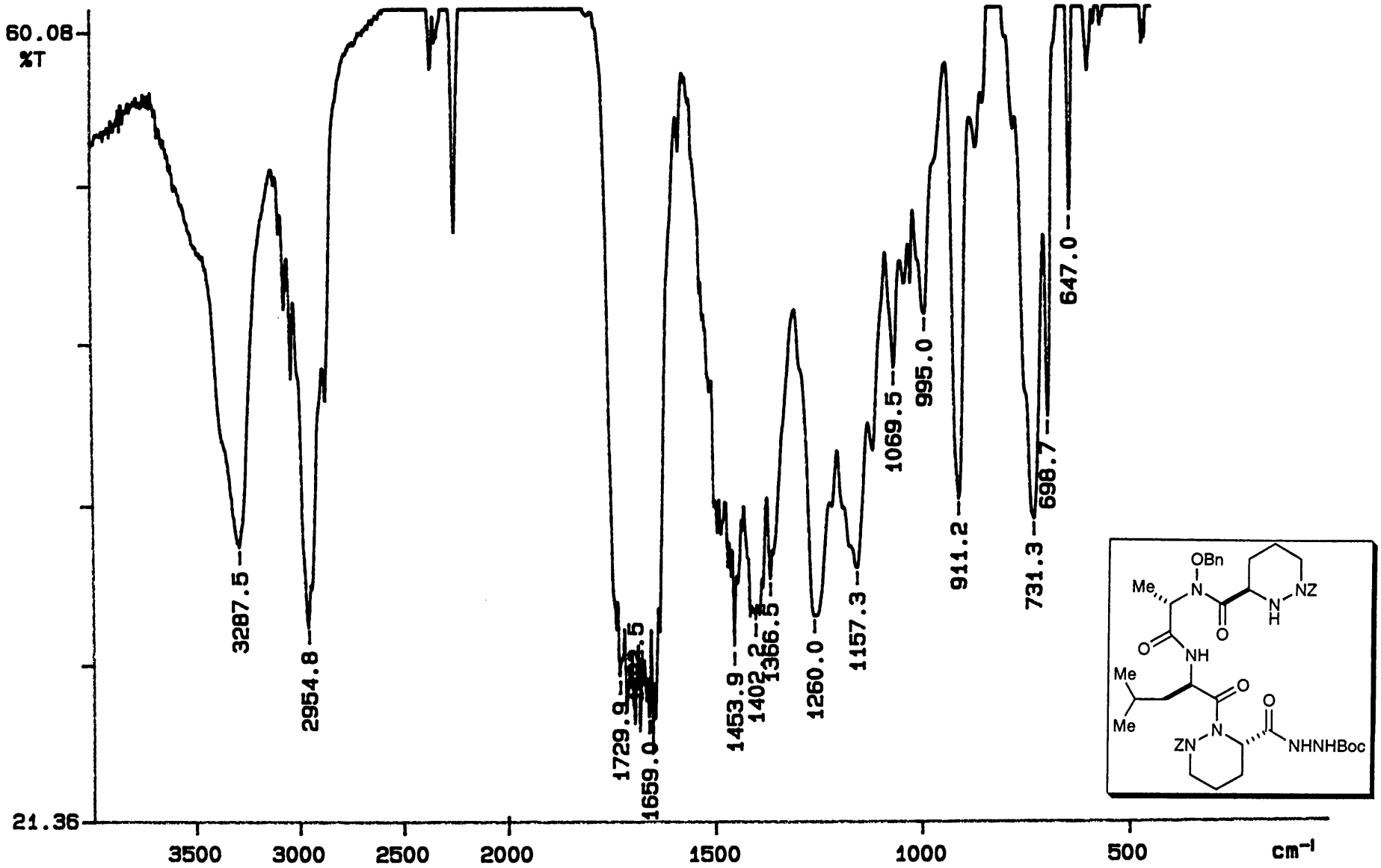

OBn
Me
N
NZ
N
H
O
O
NH
Me
O
Me
O
N
ZN
NHNHBoc

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

File:02SE571 Ident:13_17 Win 1000PPM Acq:18-FEB-2002 12:11:44 +0:54 Cal:FABLM180202_1
ZAB-SE4F FAB+ Magnet BpM:100 BpI:11156685 TIC:162890752 Flags:HALL
File Text:III-LL-69 FABMS MATRIX MNOBA + NA

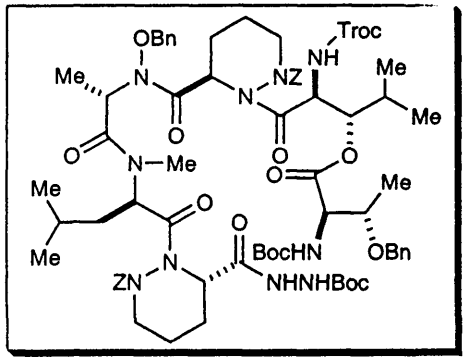


PERKIN ELMER



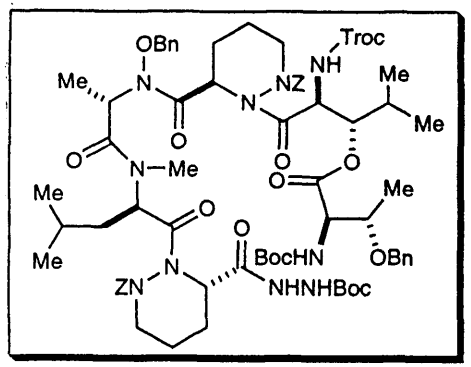
02/03/18 17:21
X: 16 scans, 4.0cm-1

III-LL-73


OBn
Troc
NH
Me
NZ
Me
Me
N–Me
Me
Me
Me
BocHN
OBn
ZN
NHNHBoc

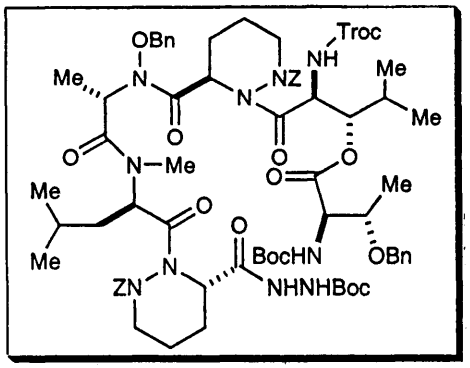
10
9
8
7
6
5
4
3
2
1
0
ppm

III-LL-73

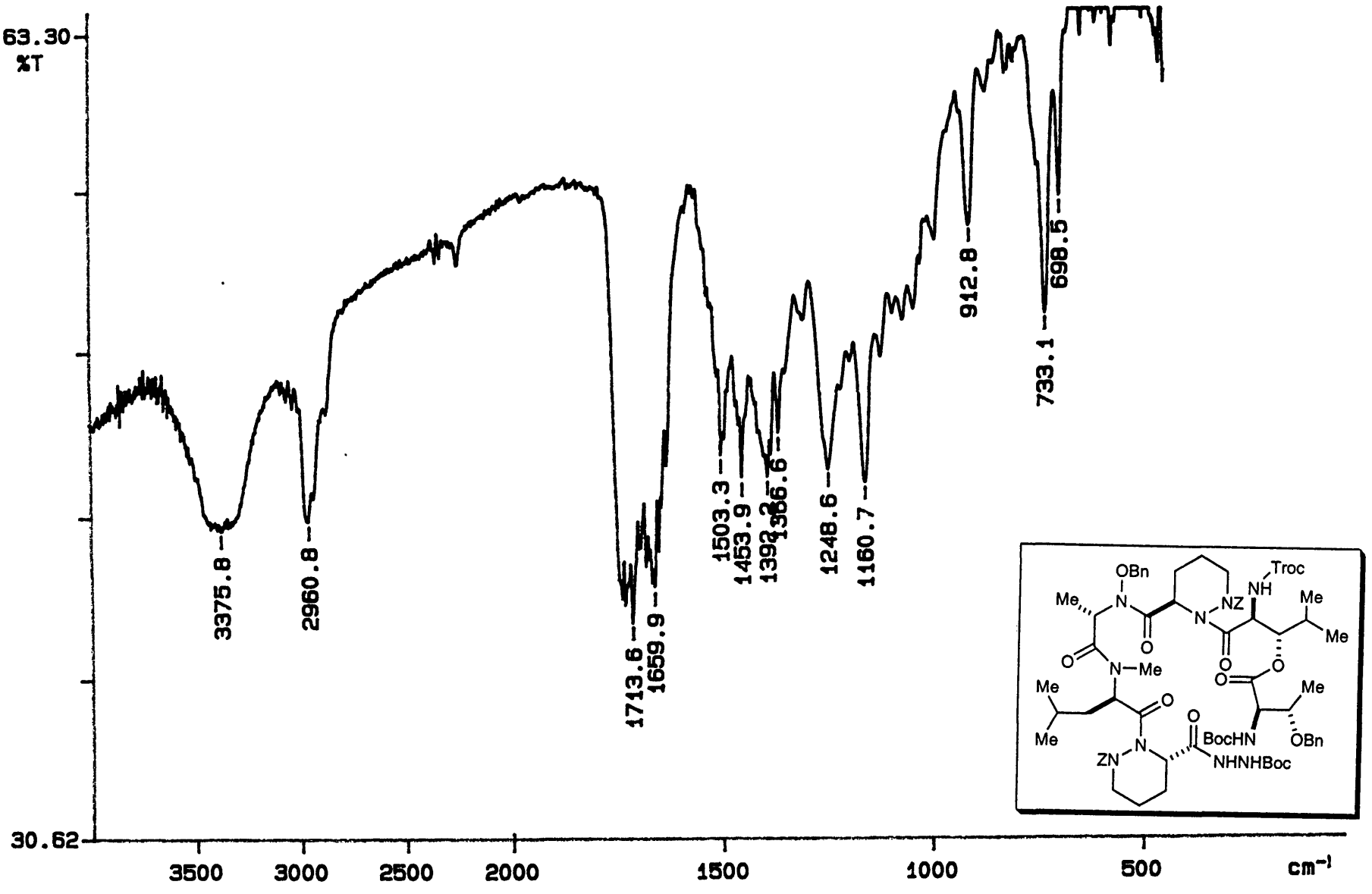

OBn
Troc
NH
Me
NZ
Me
Me
N–Me
Me
Me
Me
BocHN
OBn
ZN
NHNHBoc

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

File:02SE622 Ident:15_25 Win 1000PPM Acq:19-FEB-2002 14:45:02 +1:12 Cal:FABMM190202_1
ZAB-SE4F FAB+ Magnet BpM:175 BpI:18947352 TIC:1104965376 Flags:HALL
File Text:III-LL-73 FABMS MATRIX MNOBA + NA

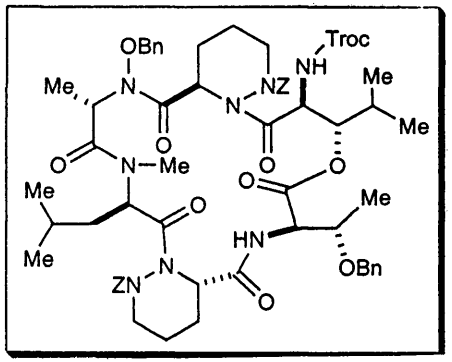


PERKIN ELMER



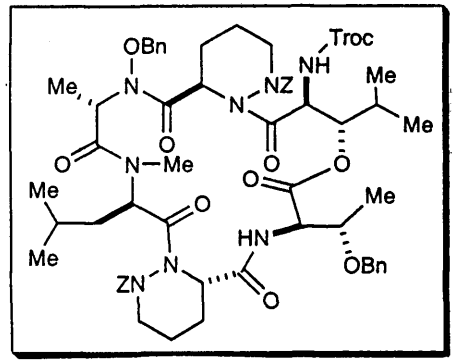
02/03/18 17:31
X: 16 scans, 4.0cm-1

III-LL-75

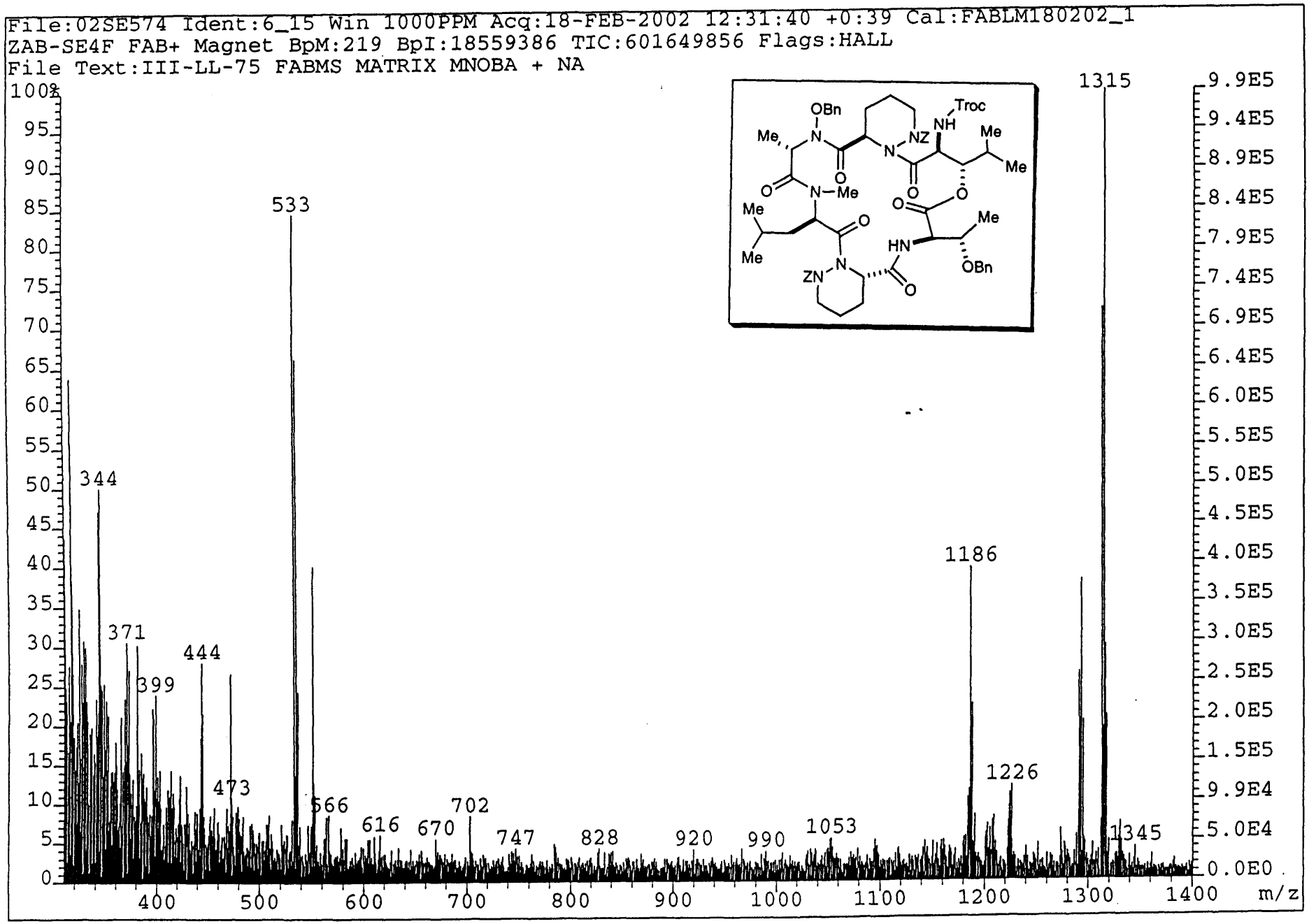

OBn
Troc
NH
Me
Me
NZ
N
Me
O
N–Me
O
O
O
Me
Me
Me
HN
OBn
ZN
N
O

10
9
8
7
6
5
4
3
2
1
0
ppm

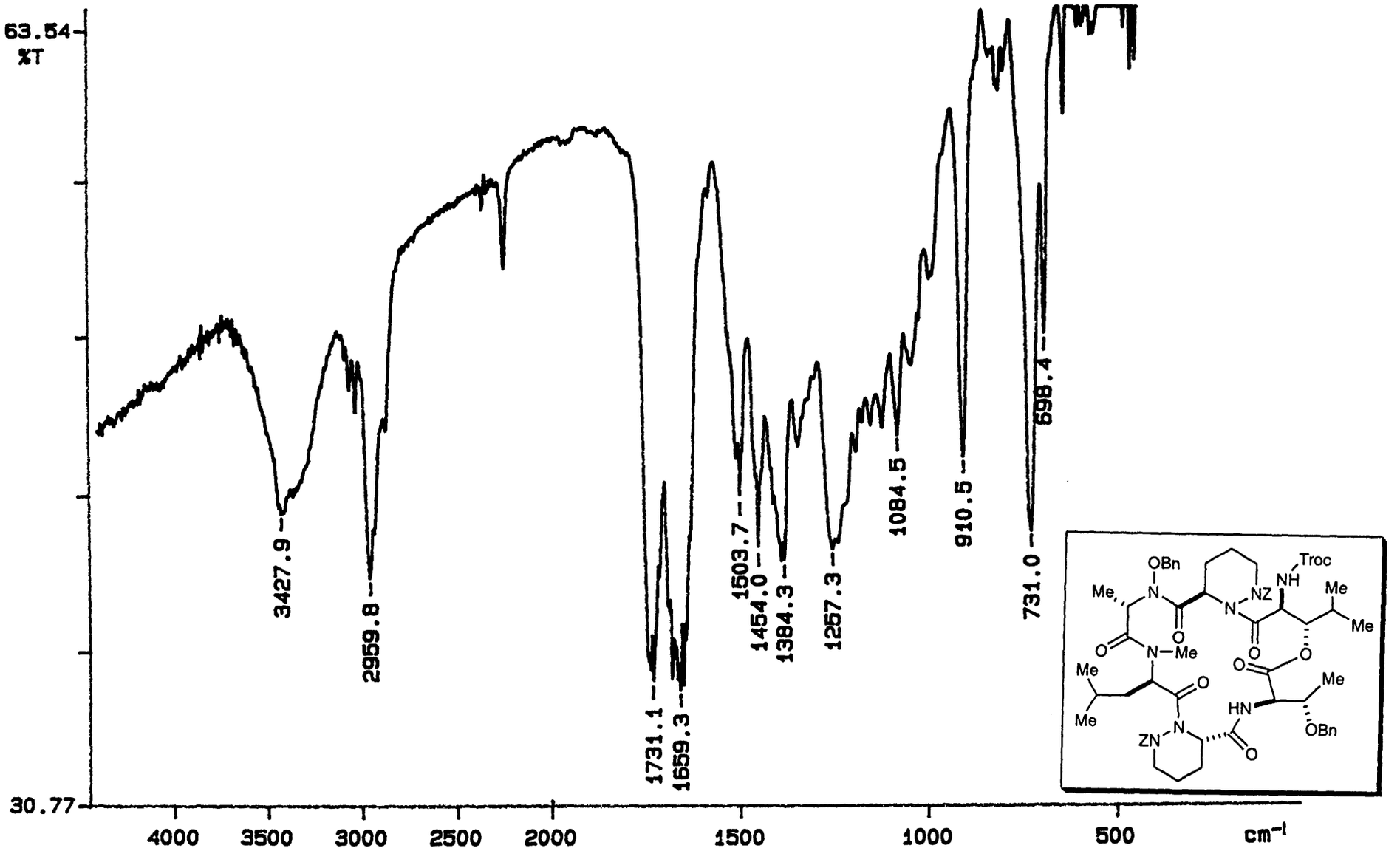
III-LL-75


OBn
Troc
NH
Me
Me,
N
NZ
N
Me
O
O
N–Me
O
O
O
Me
Me
O
Me
HN
Me
OBn
ZN
N
O

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

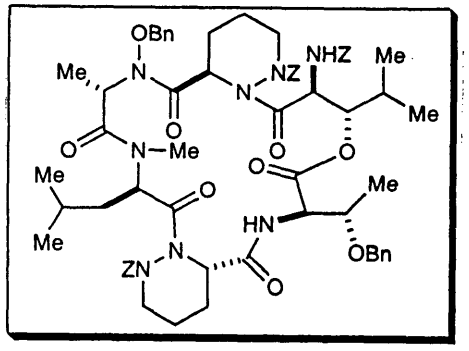

File:02SE574 Ident:6_15 Win 1000PPM Acq:18-FEB-2002 12:31:40 +0:39 Cal:FABLM180202_1
ZAB-SE4F FAB+ Magnet BpM:219 BpI:18559386 TIC:601649856 Flags:HALL
File Text:III-LL-75 FABMS MATRIX MNOBA + NA
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
9.9E5
9.4E5
8.9E5
8.4E5
7.9E5
7.4E5
6.9E5
6.4E5
6.0E5
5.5E5
5.0E5
4.5E5
4.0E5
3.5E5
3.0E5
2.5E5
2.0E5
1.5E5
9.9E4
5.0E4
0.0E0
344
371
399
444
473
533
566
616
670
702
747
828
920
990
1053
1186
1226
1315
1345
400
500
600
700
800
900
1000
1100
1200
1300
1400
m/z
OBn
Troc
NH
NZ
Me
N-Me
HN
ZN
O

PERKIN ELMER



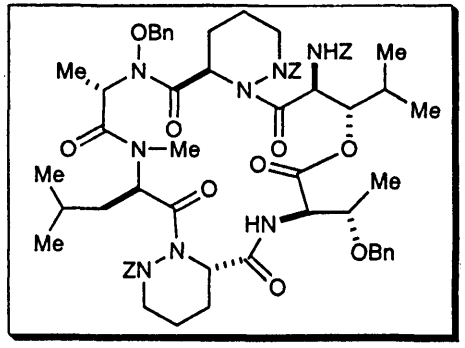
02/03/18 17:42
X: 16 scans, 4.0cm-1

III-LL-123

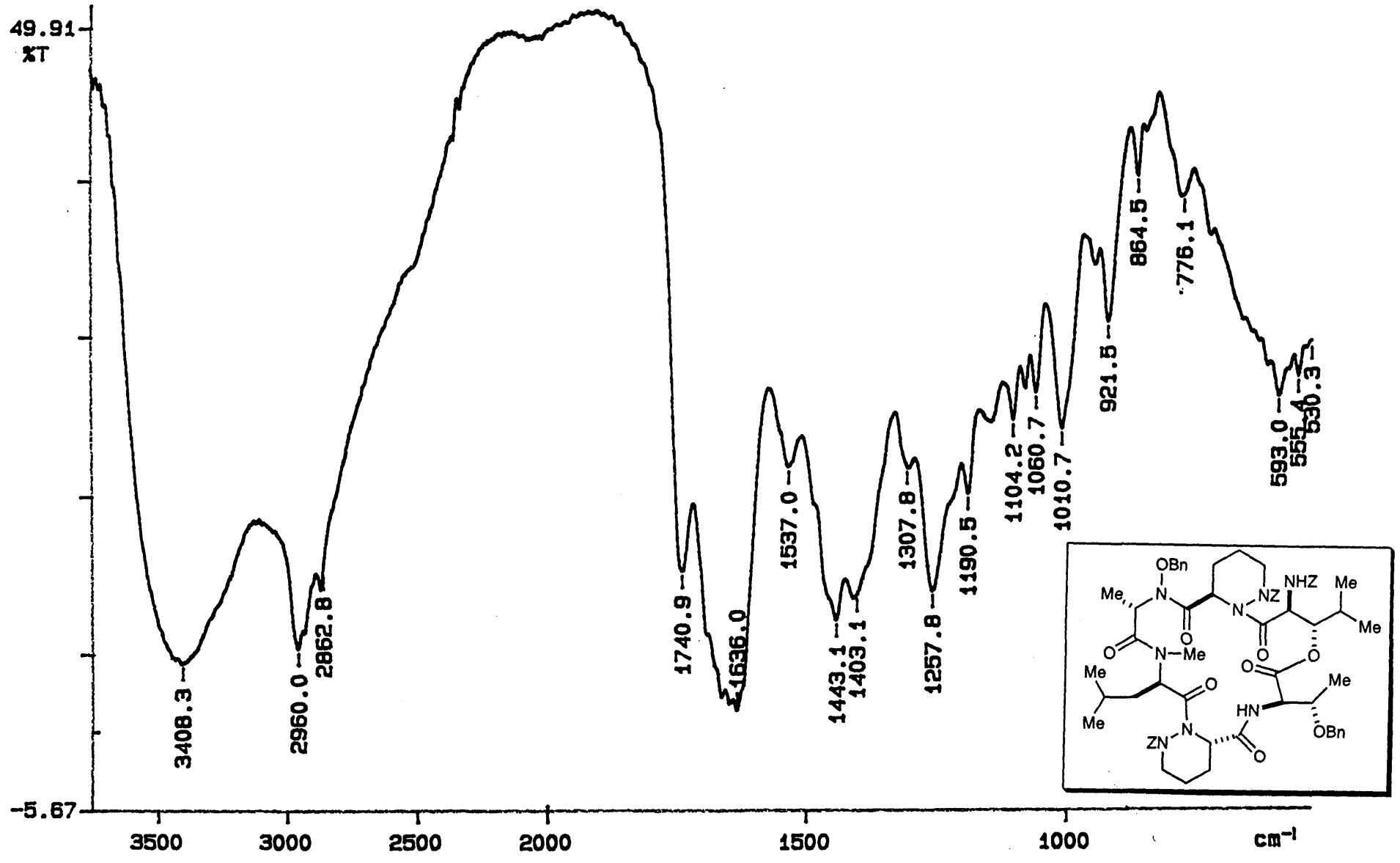

OBn
Me
N
NHZ
Me
NZ
N
Me
O
O
N–Me
O
O
O
Me
Me
O
Me
HN
Me
OBn
ZN
N
O

10
9
8
7
6
5
4
3
2
1
0
ppm

File:02SE573 Ident:58 Acq:18-FEB-2002 12:24:46 +3:18 Cal:FABLM180202_1
ZAB-SE4F FAB+ Magnet BpI:15719198 TIC:377008832 Flags:HALL
File Text:III-LL-123 FABMS MATRIX MNOBA + NA

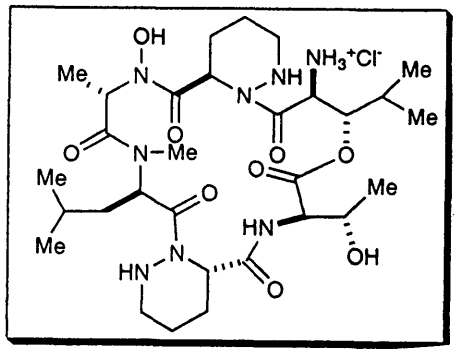


PERKIN ELMER



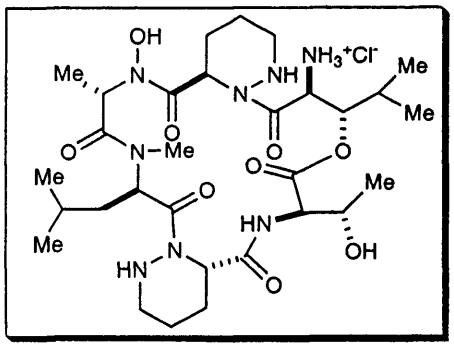
02/03/14 14:35
X: 16 scans, 16.0cm-1, apod none

III-LL-124


OH
Me
N
NH
NH3+Cl-
Me
Me
O
N–Me
O
O
O
Me
Me
O
Me
HN
OH
HN
N
O

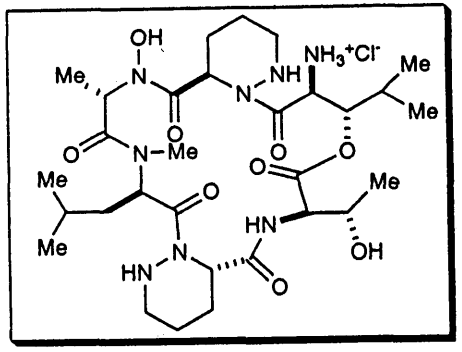
10
9
8
7
6
5
4
3
2
1
0
ppm

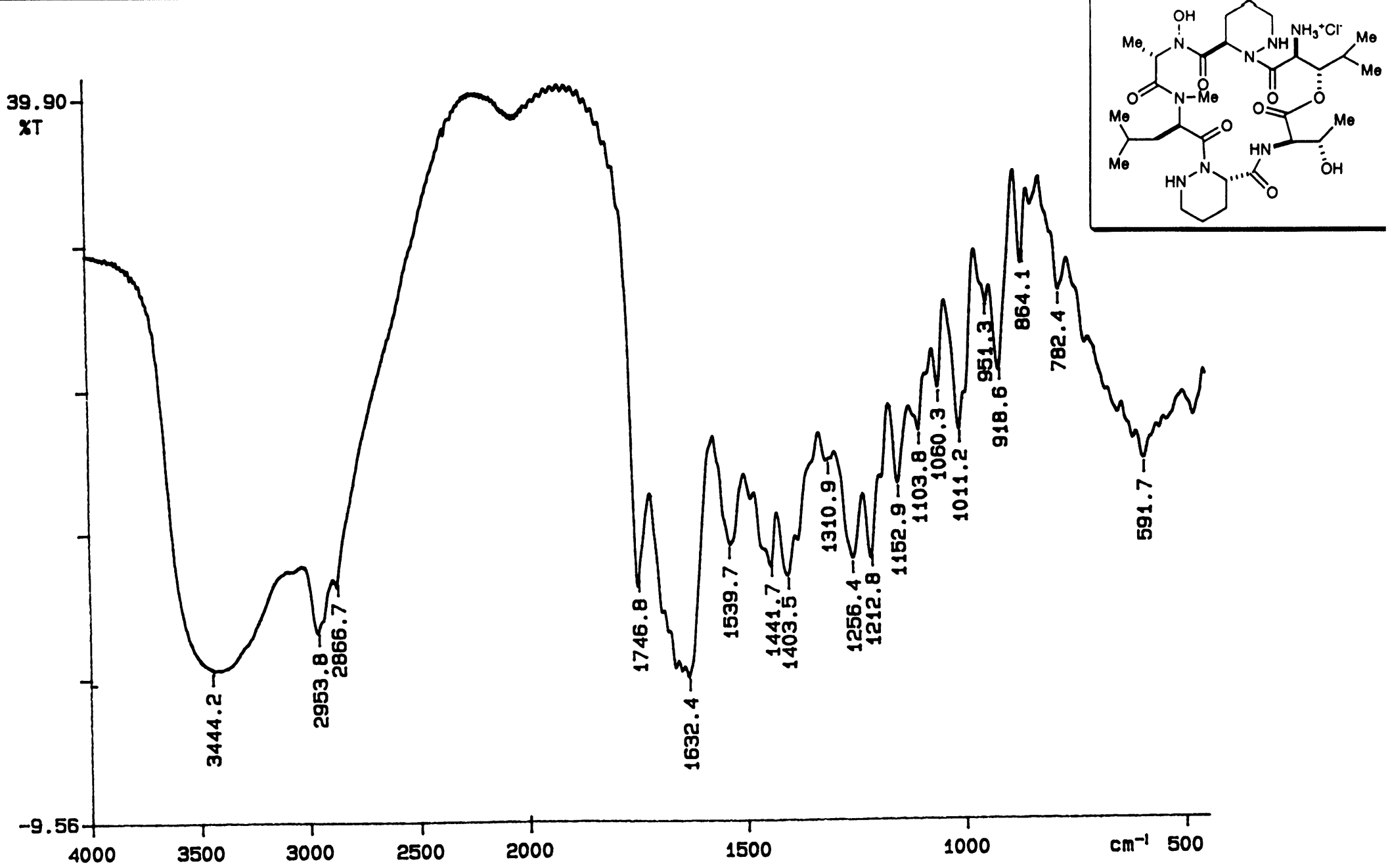
III-LL-124


OH
Me
N
NH
NH3+Cl-
Me
Me
O
N-Me
O
O
O
Me
Me
O
Me
HN
Me
Me
N
OH
HN
O

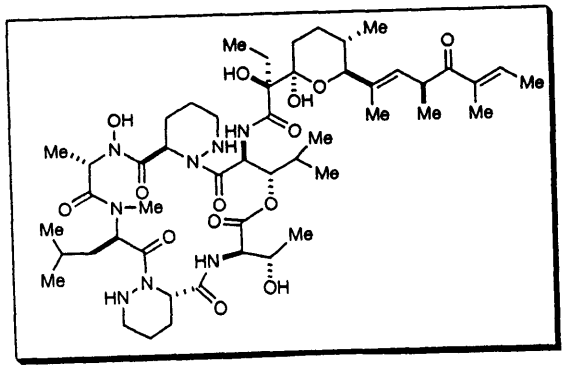
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

File:02SE568 Ident:10 Acq:18-FEB-2002 11:17:41 +0:37 Cal:FABLM180202_1
ZAB-SE4F FAB+ Magnet BpI:10007478 TIC:197977968 Flags:HALL
File Text:III-LL-124 FABMS MATRIX MNOBA + NA

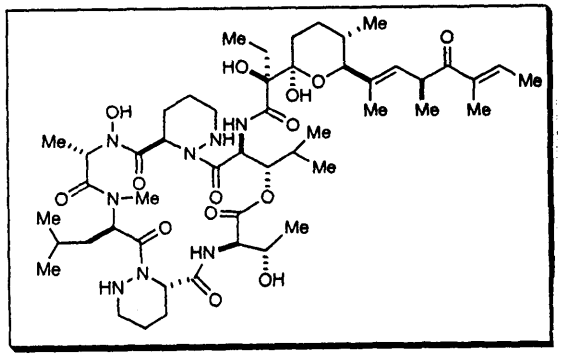



39.90
%T
-9.56
4000
3500
3000
2500
2000
1500
1000
cm⁻¹
500
3444.2
2953.8
2866.7
1746.8
1632.4
1539.7
1441.7
1403.5
1310.9
1256.4
1212.8
1152.9
1103.8
1060.3
1011.2
951.3
918.6
864.1
782.4
591.7
OH
Me
N
NH
NH3+Cl-
Me
Me
O
N-Me
O
O
O
Me
Me
O
HN
OH
HN
N
O

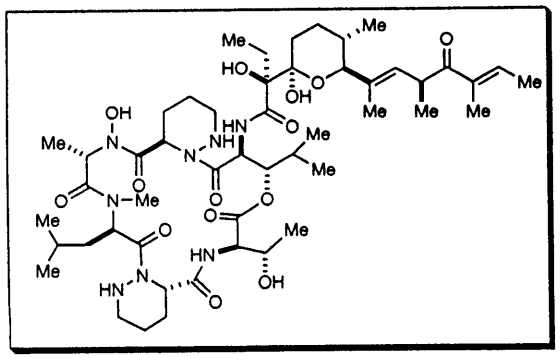
500MHz PROTON
In CDCl3 298K

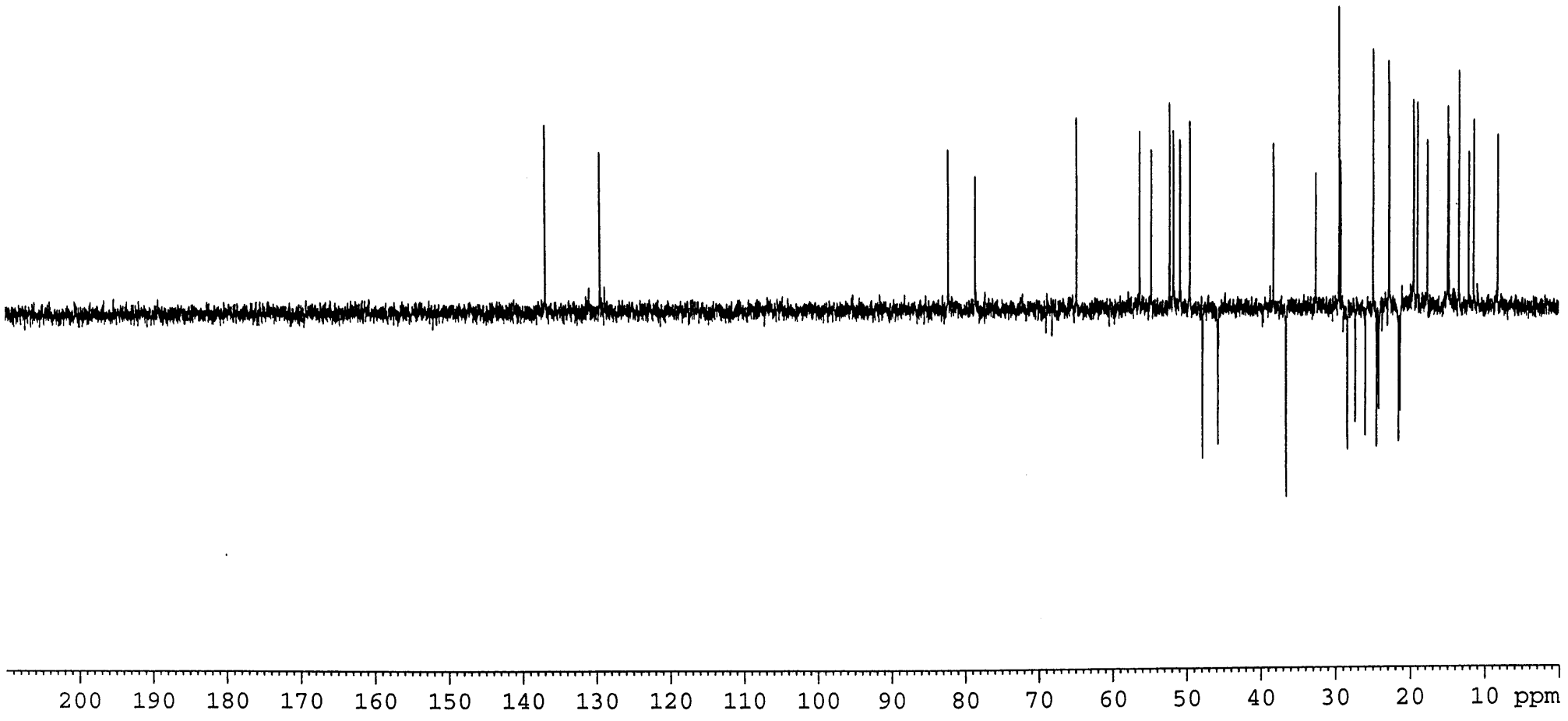


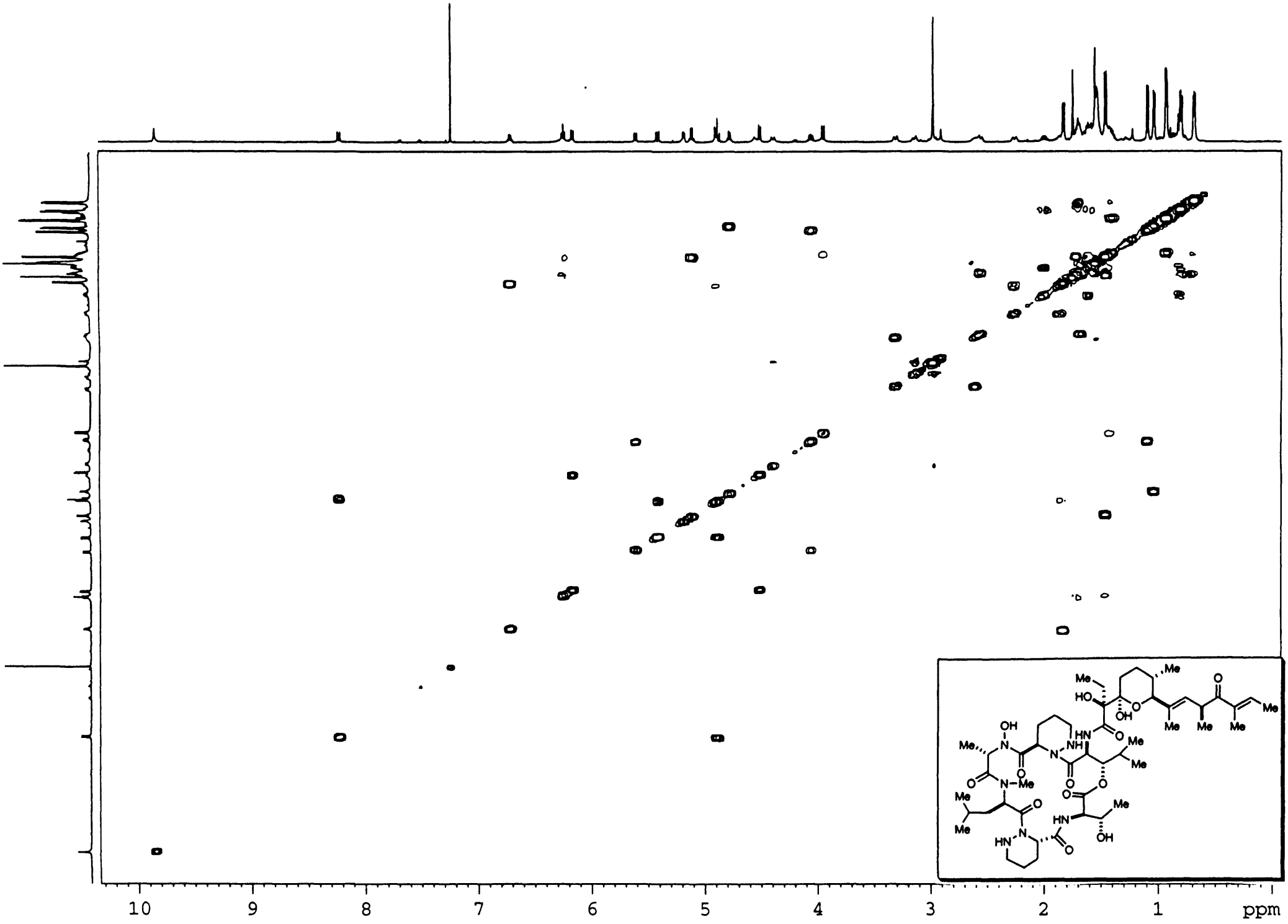
125MHz CNMR
298K In CDCl3



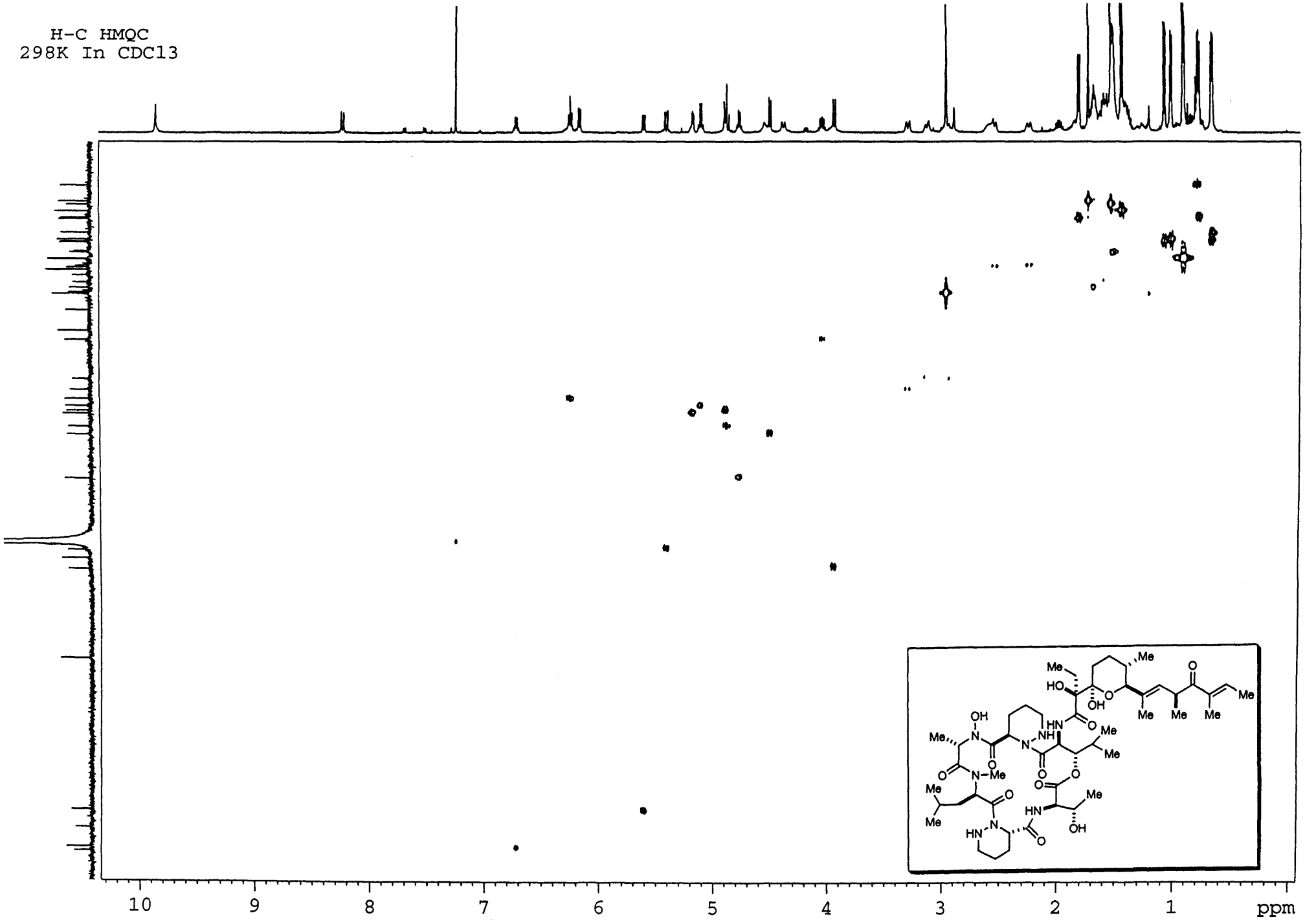
C DEPT
298K In CDCl3

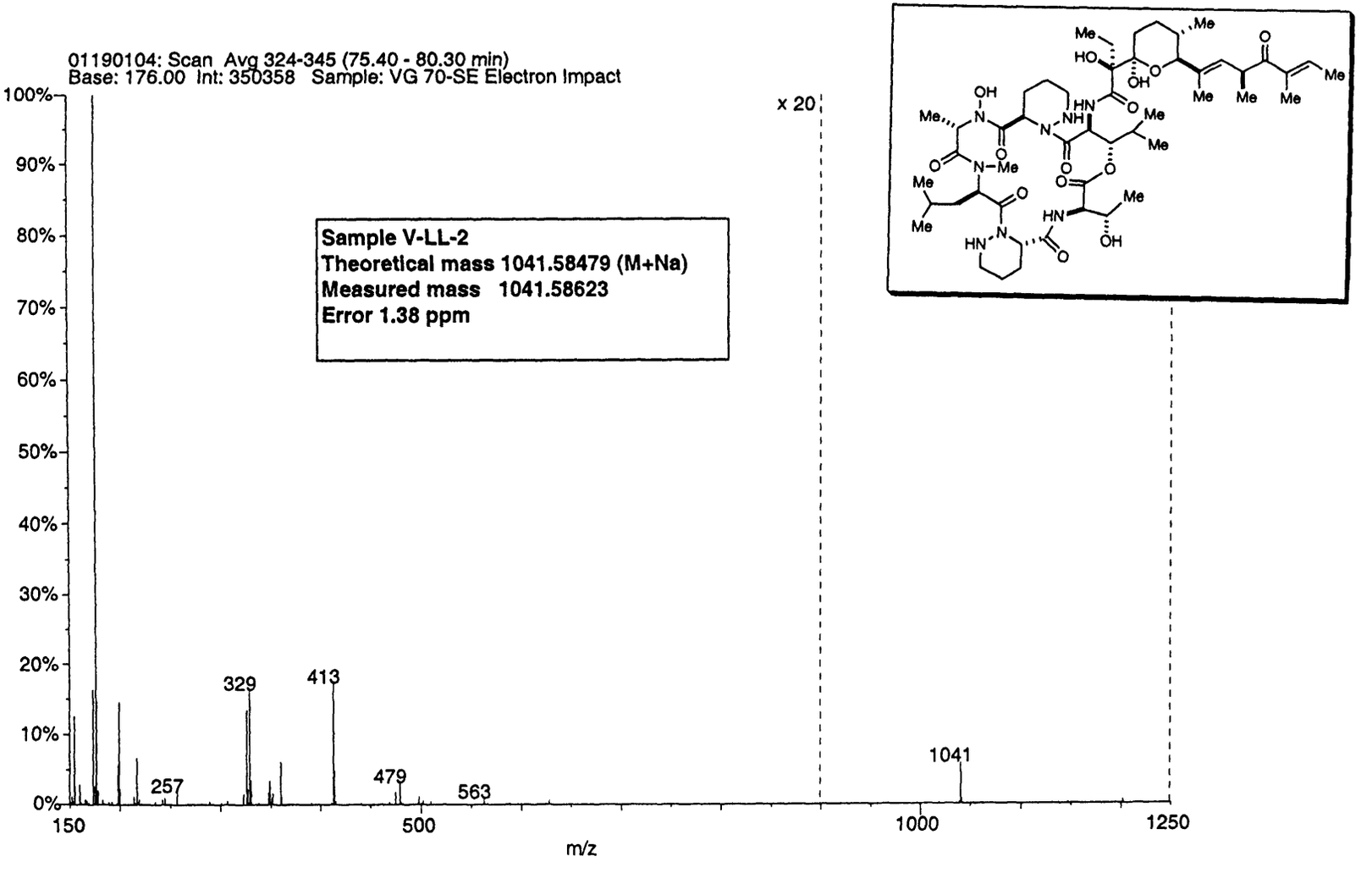


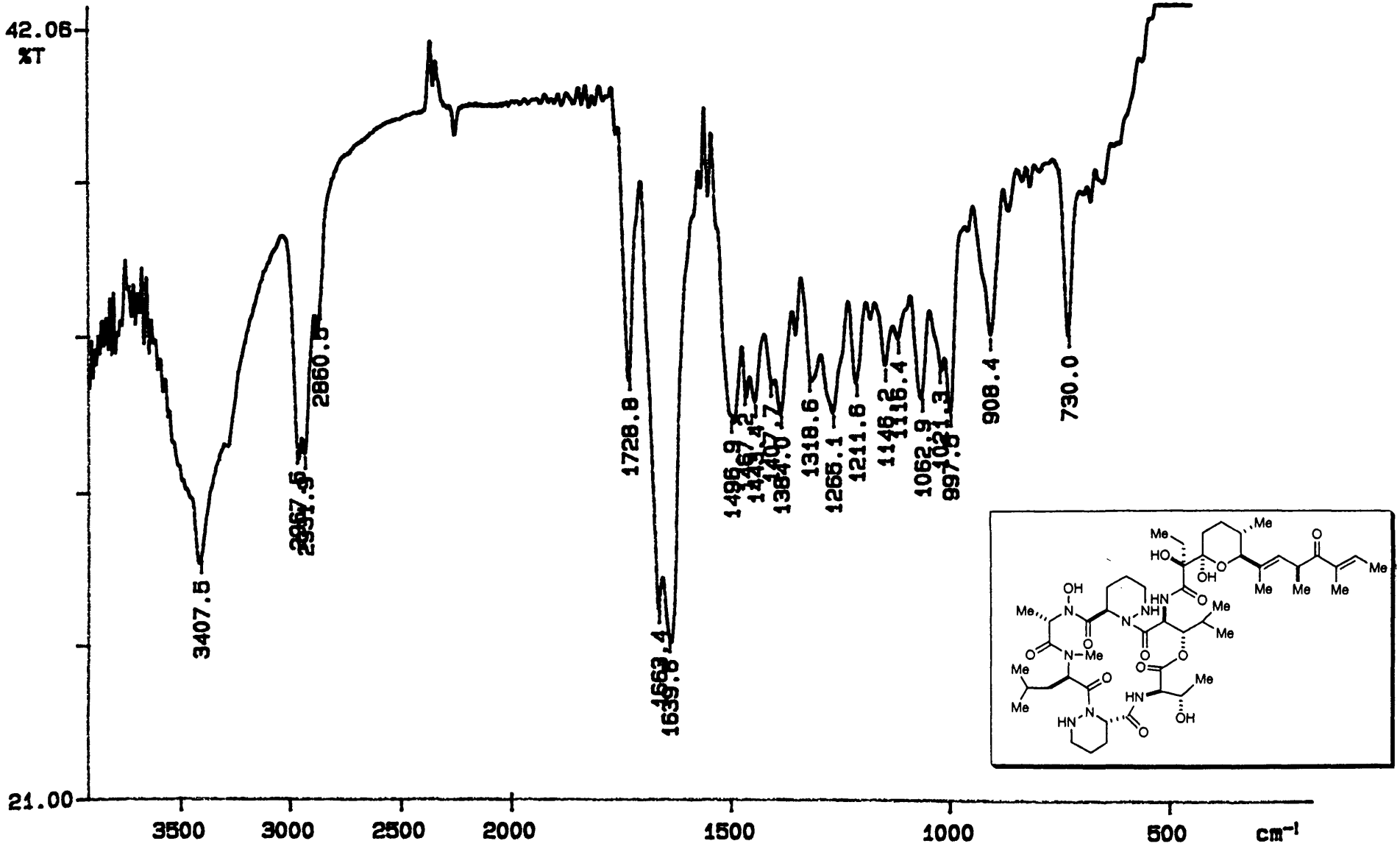



10
9
8
7
6
5
4
3
2
1
ppm

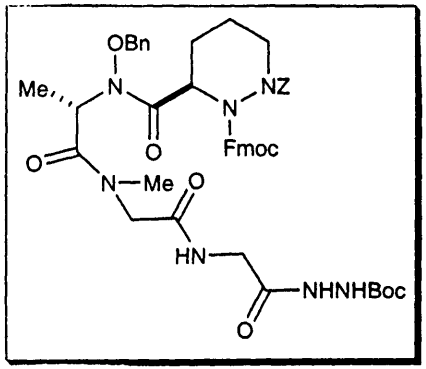
H-C HMQC
298K In CDC13



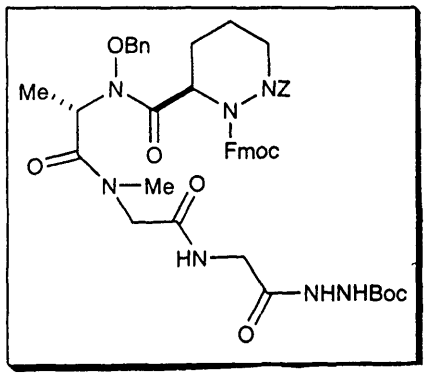

01190104: Scan Avg 324-345 (75.40 - 80.30 min)
Base: 176.00 Int: 350358 Sample: VG 70-SE Electron Impact
Sample V-LL-2
Theoretical mass 1041.58479 (M+Na)
Measured mass 1041.58623
Error 1.38 ppm
x 20
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
150
500
1000
1250
m/z
257
329
413
479
563
1041


42.06
%T
21.00
3407.5
2967.6
2931.5
2860.5
1728.8
1639.8
1496.9
1467.2
1444.4
1384.0
1318.6
1265.1
1211.8
1148.2
1116.4
1062.9
1021.3
997.8
908.4
730.0
3500
3000
2500
2000
1500
1000
500
cm-1

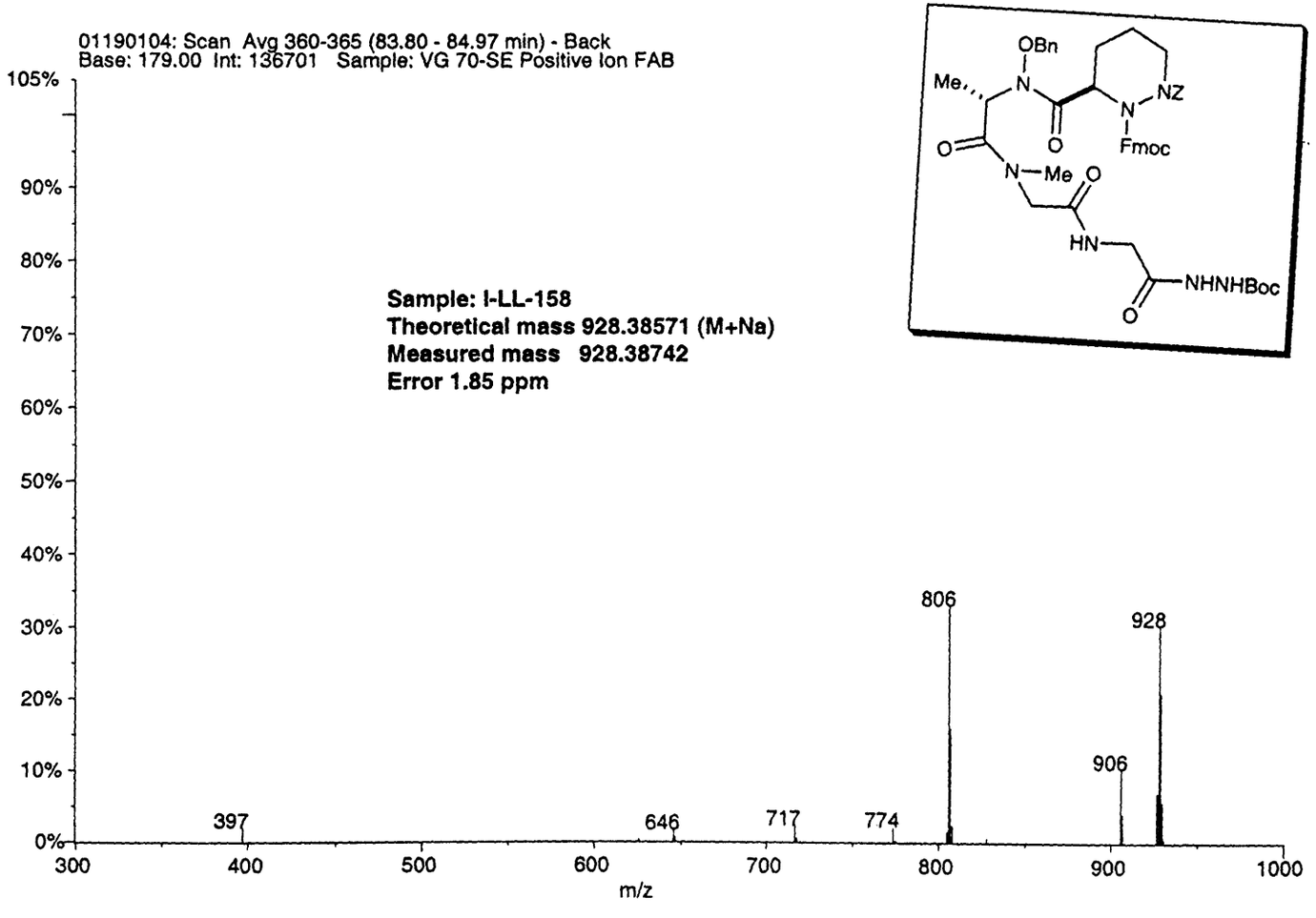
I-LL-158

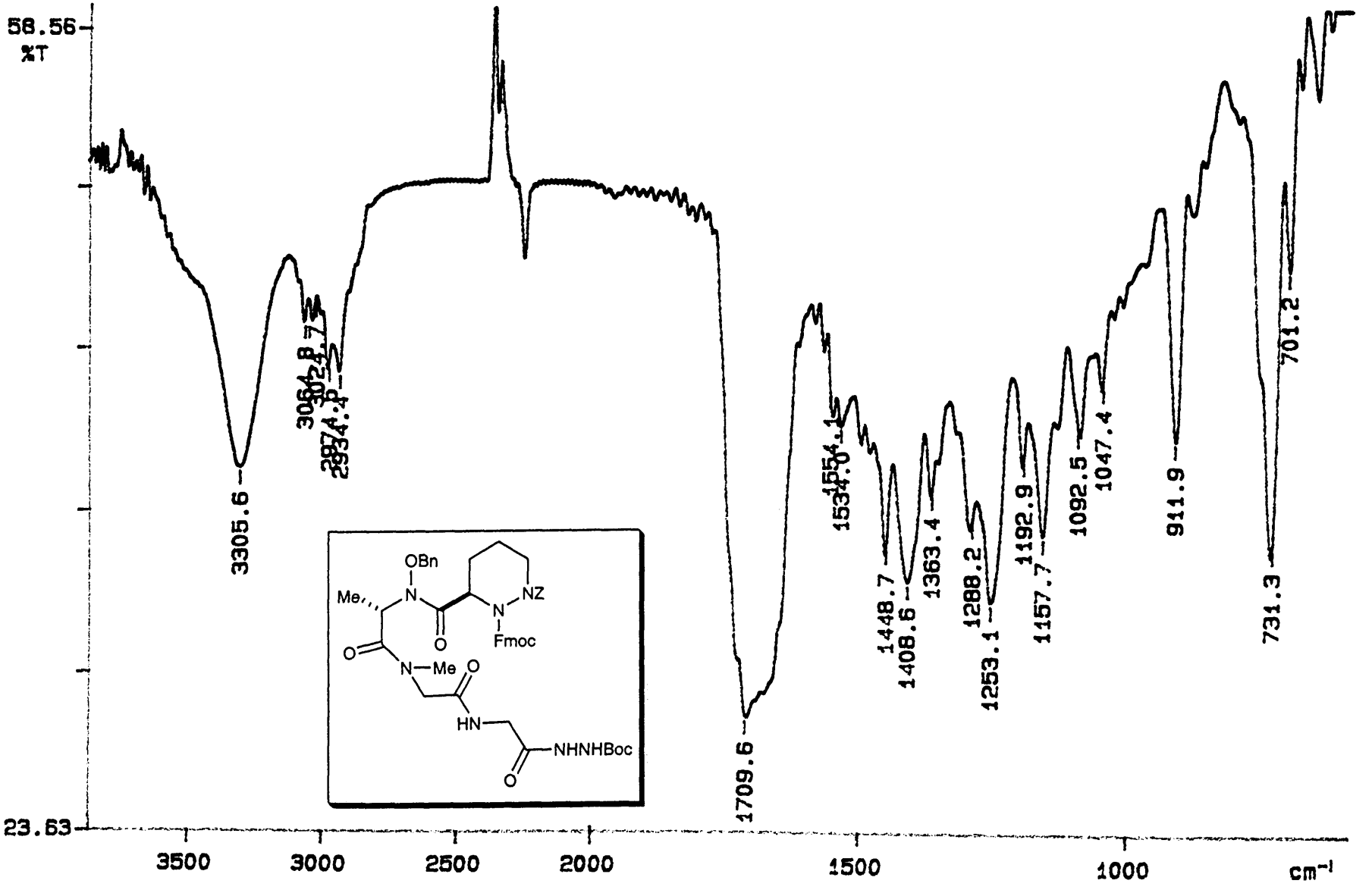


I-LL-158

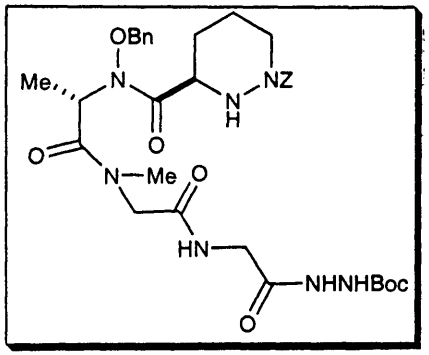

OBn
Me
N
NZ
N
Fmoc
O
O
N—Me
O
HN
NHNHBoc
O

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

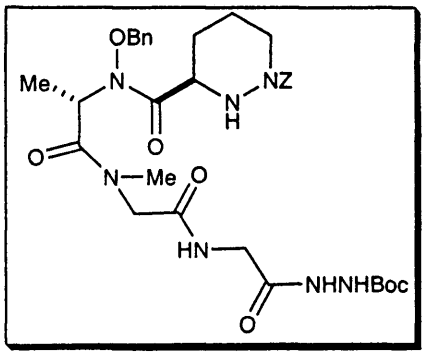

01190104: Scan Avg 360-365 (83.80 - 84.97 min) - Back
Base: 179.00 Int: 136701 Sample: VG 70-SE Positive Ion FAB
Sample: I-LL-158
Theoretical mass 928.38571 (M+Na)
Measured mass 928.38742
Error 1.85 ppm
OBn
Me
N
NZ
N
Fmoc
O
O
N—Me
O
HN
NHNHBoc
O
105%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
397
646
717
774
806
906
928
300
400
500
600
700
800
900
1000
m/z


58.56
%T
23.63
3305.6
3064.8
3024.7
2934.4
1709.6
1554.1
1534.0
1448.7
1408.6
1363.4
1288.2
1253.1
1192.9
1157.7
1092.5
1047.4
911.9
731.3
701.2
3500
3000
2500
2000
1500
1000
cm⁻¹
OBn
Me
N
NZ
Fmoc
O
N—Me
HN
NHNHBoc

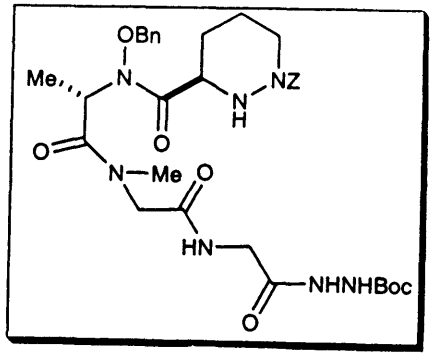
I-LL-159

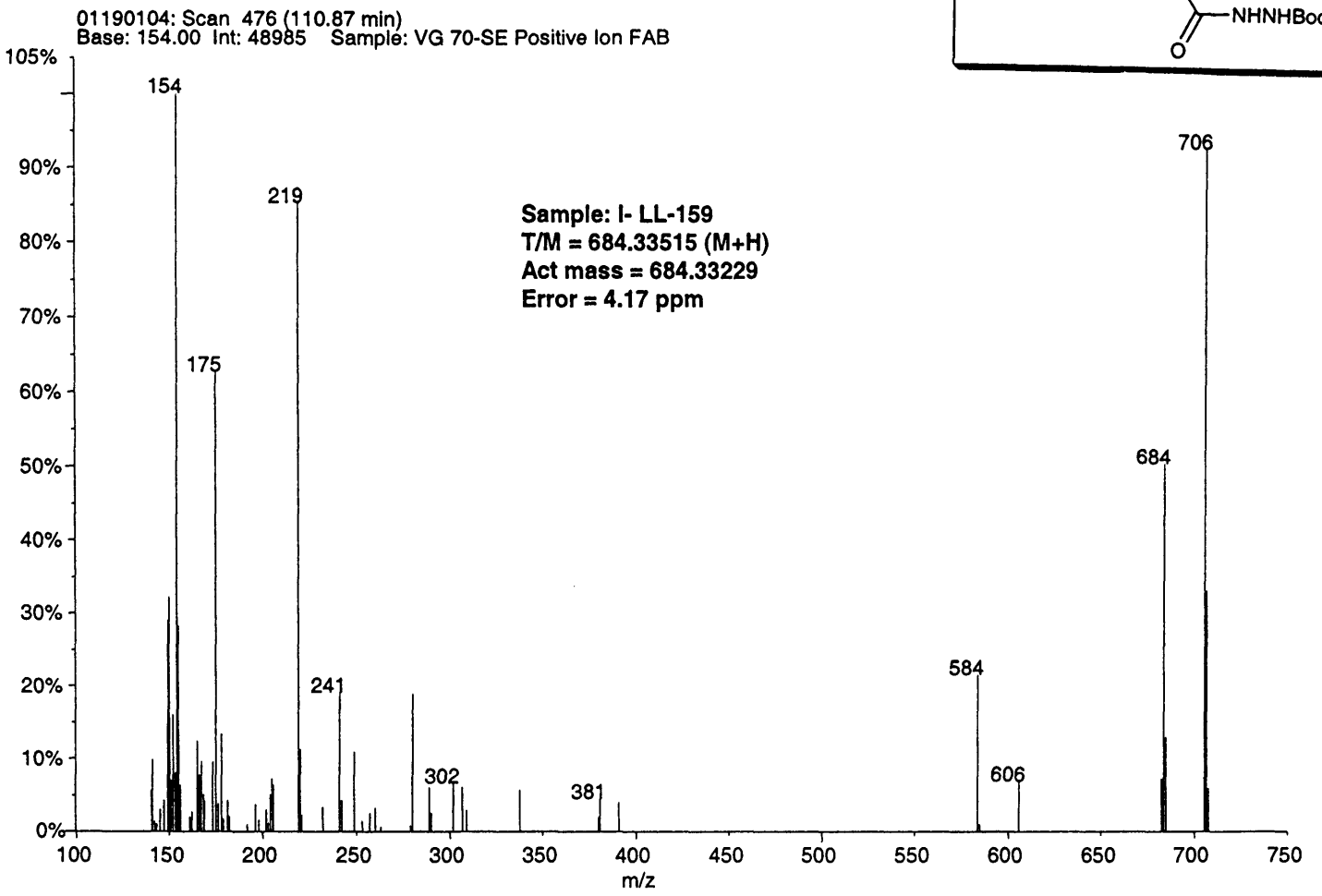


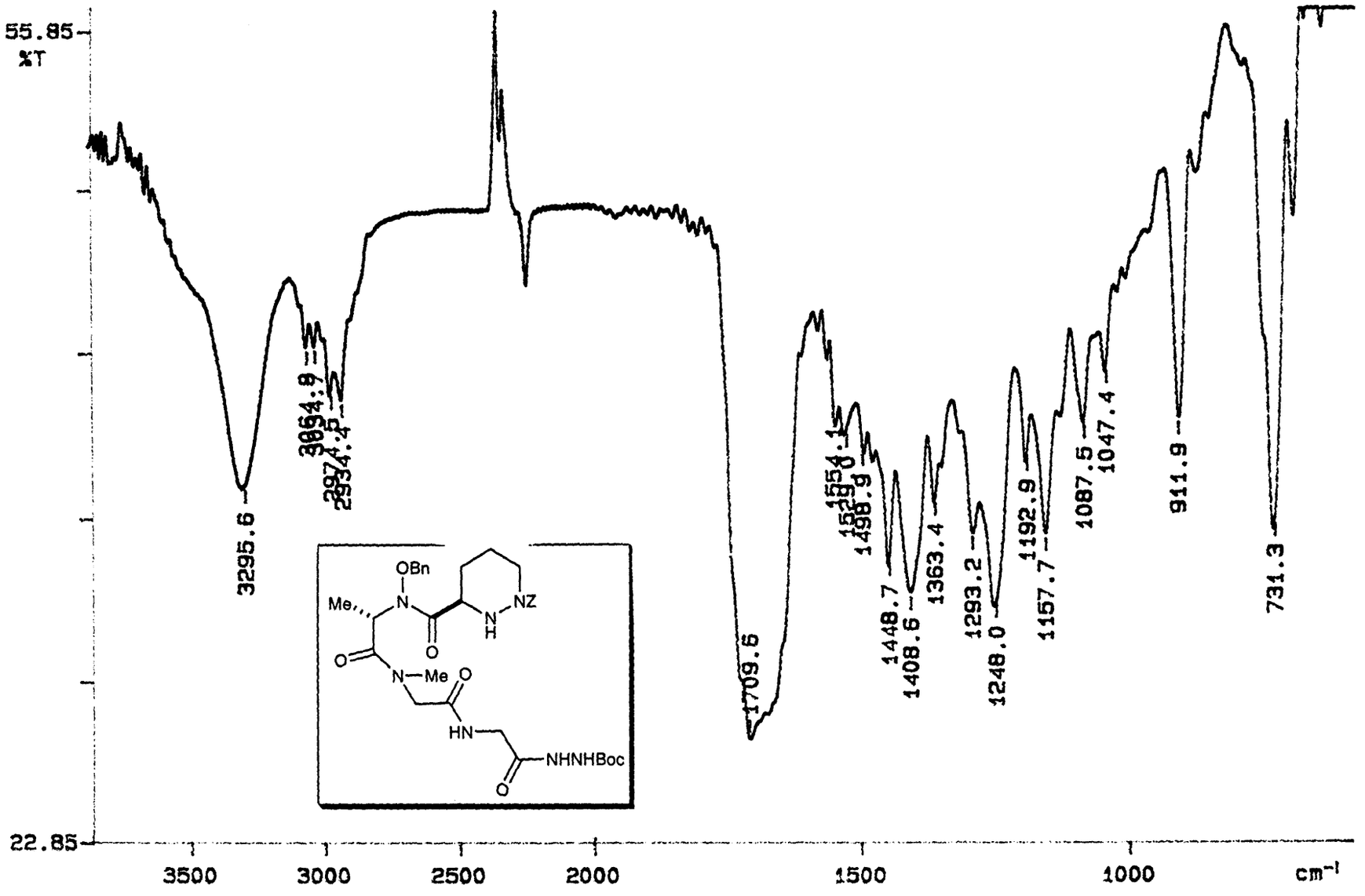
I-LL-159


OBn
N
Me
NZ
N
H
O
O
N—Me
O
HN
NHNHBoc
O

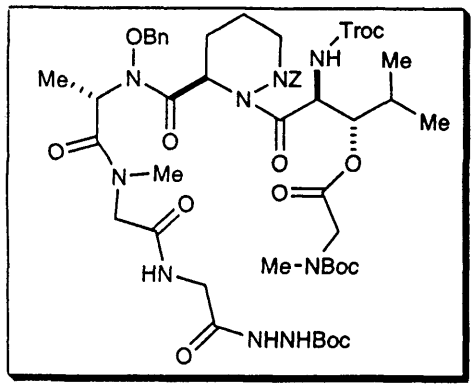
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


OBn
Me
N
NZ
N
H
O
O
N
Me
O
HN
NHNHBoc
O


01190104: Scan 476 (110.87 min)
Base: 154.00 Int: 48985 Sample: VG 70-SE Positive Ion FAB
Sample: I- LL-159
T/M = 684.33515 (M+H)
Act mass = 684.33229
Error = 4.17 ppm
105%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
154
219
175
241
302
381
584
606
684
706
100
150
200
250
300
350
400
450
500
550
600
650
700
750
m/z

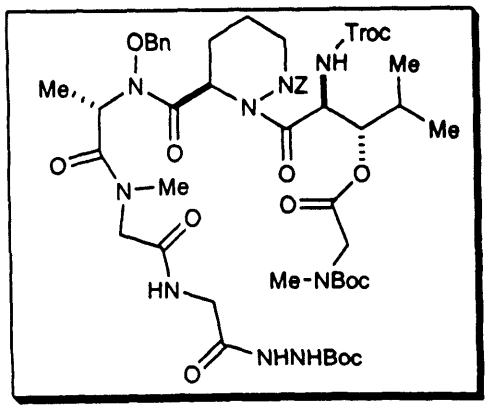

55.85
%T
22.85
3500
3000
2500
2000
1500
1000
cm⁻¹
3295.6
3064.9
3054.5
2974.4
2934.5
1709.6
1554.1
1529.0
1498.9
1448.7
1408.6
1363.4
1293.2
1248.0
1192.9
1157.7
1087.5
1047.4
911.9
731.3
OBn
Me
N
NZ
H
O
N—Me
HN
NHNHBoc

I-LL-160

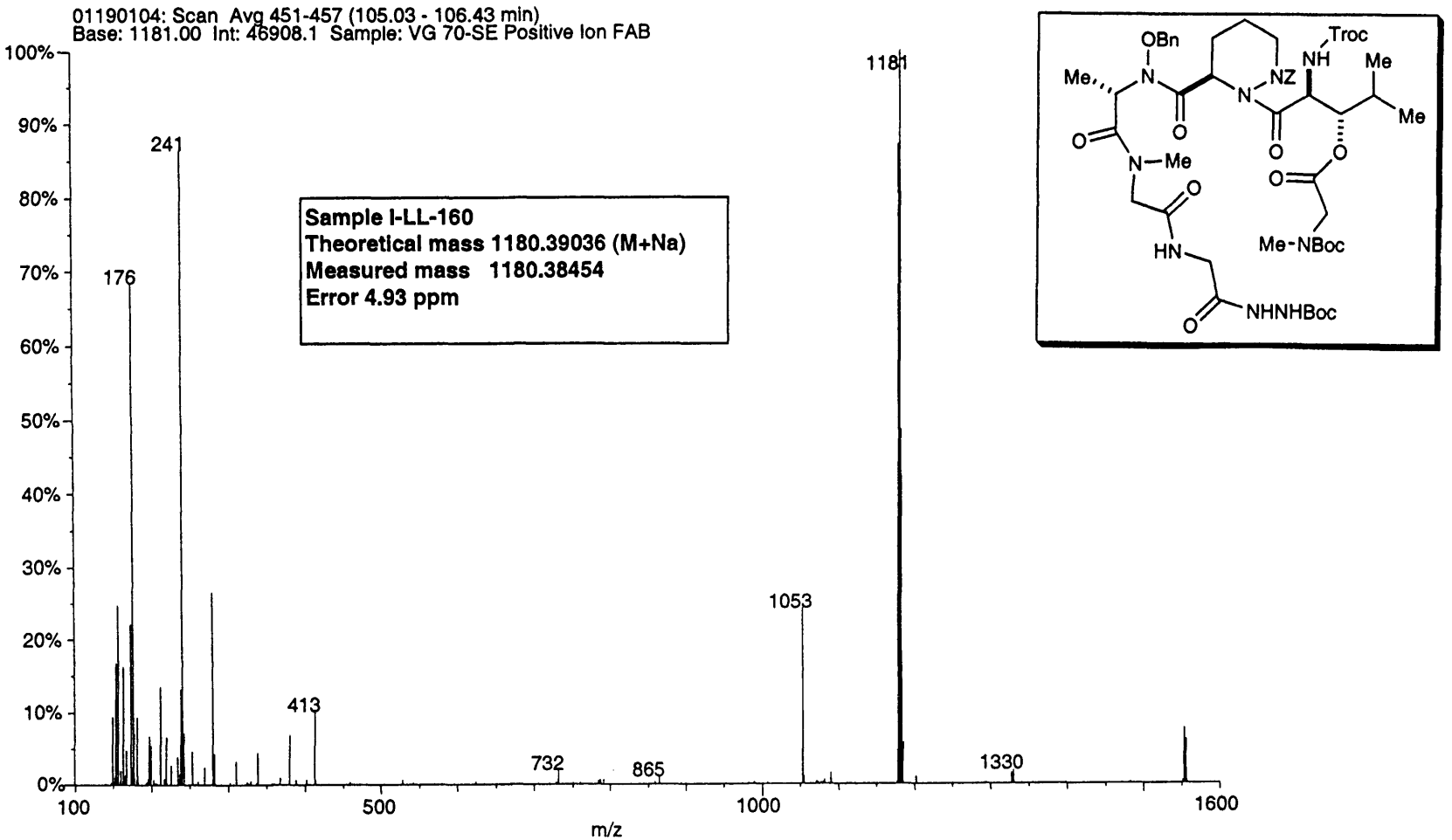

OBn
Troc
NH
Me
Me
N
NZ
N
Me
O
O
O
O
N–Me
O
O
HN
Me-NBoc
NHNHBoc
O

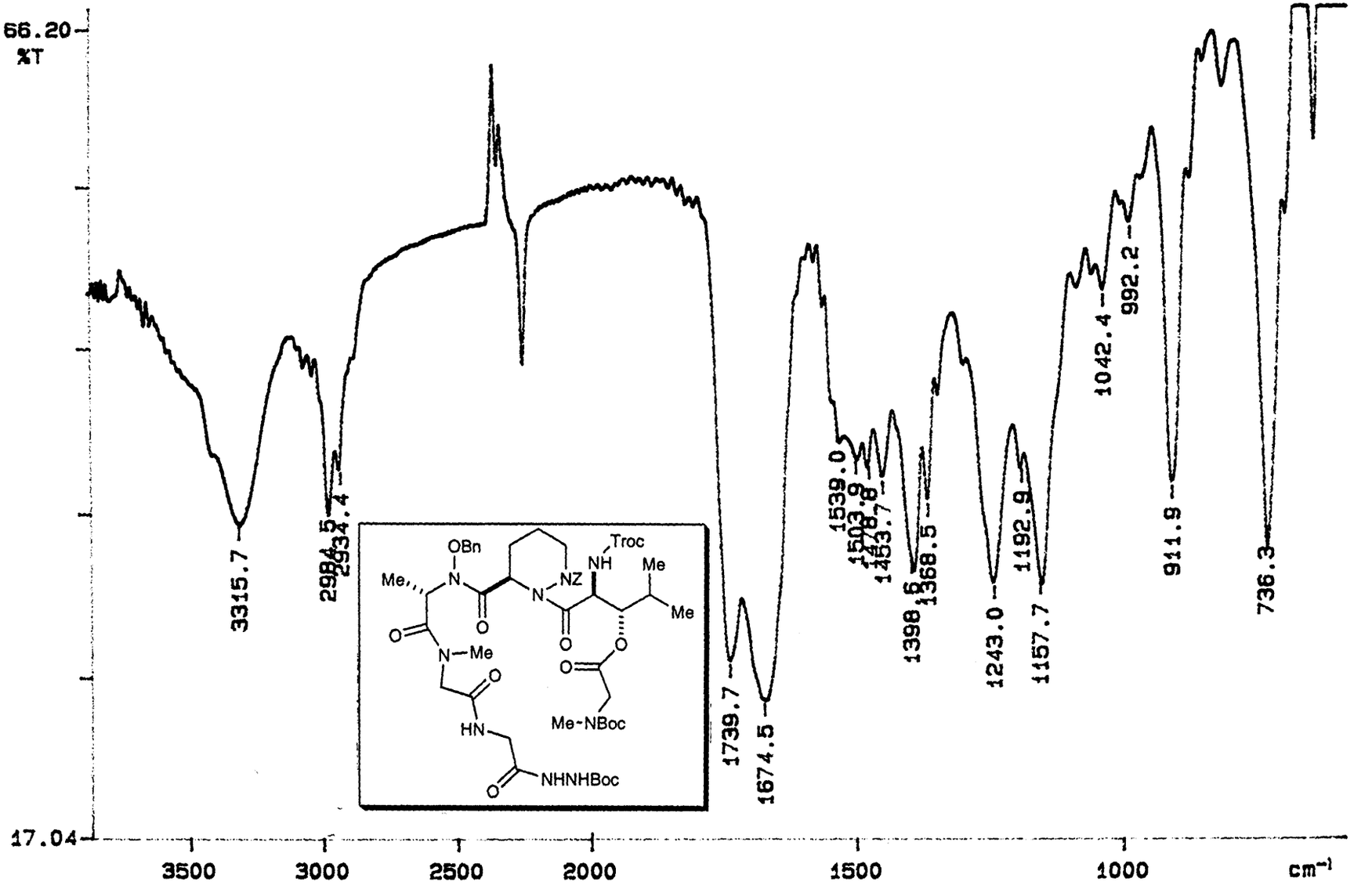
9
8
7
6
5
4
3
2
1
0
ppm

I-LL-160

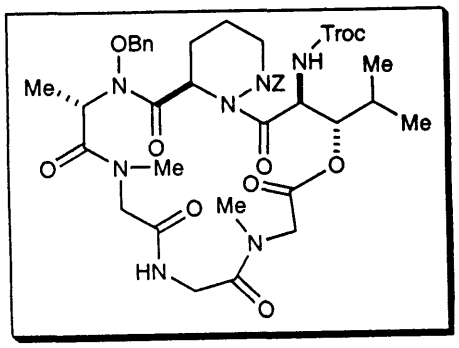

OBn
Troc
NH
Me
Me
NZ
N
N
Me
O
O
O
O
O
N–Me
O
HN
Me-NBoc
O
NHNHBoc

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

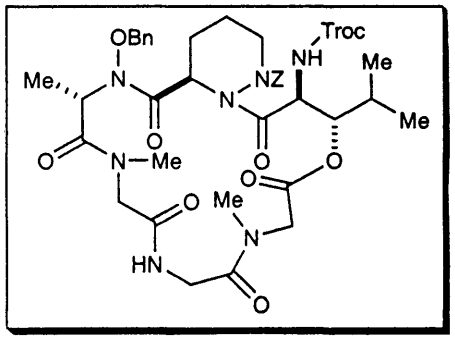

01190104: Scan Avg 451-457 (105.03 - 106.43 min)
Base: 1181.00 Int: 46908.1 Sample: VG 70-SE Positive Ion FAB
Sample I-LL-160
Theoretical mass 1180.39036 (M+Na)
Measured mass 1180.38454
Error 4.93 ppm
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100
500
1000
1600
m/z
241
176
413
732
865
1053
1181
1330
OBn
Troc
NH
Me
Me
NZ
N
N
Me
O
N–Me
Me-NBoc
HN
NHNHBoc


66.20
%T
17.04
3315.7
2934.4
1739.7
1674.5
1539.0
1503.9
1478.8
1453.7
1398.6
1368.5
1243.0
1192.9
1157.7
1042.4
992.2
911.9
736.3
3500
3000
2500
2000
1500
1000
cm-1
OBn
Troc
NH
Me
NZ
Me
N
Me
N
Me
HN
Me-NBoc
NHNHBoc

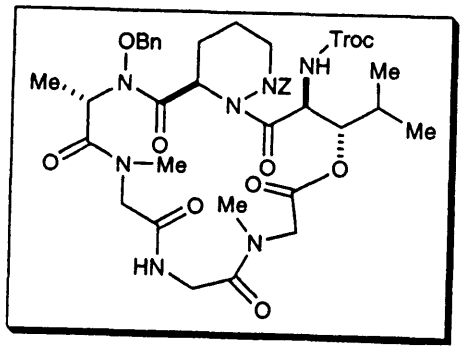
I-LL-162

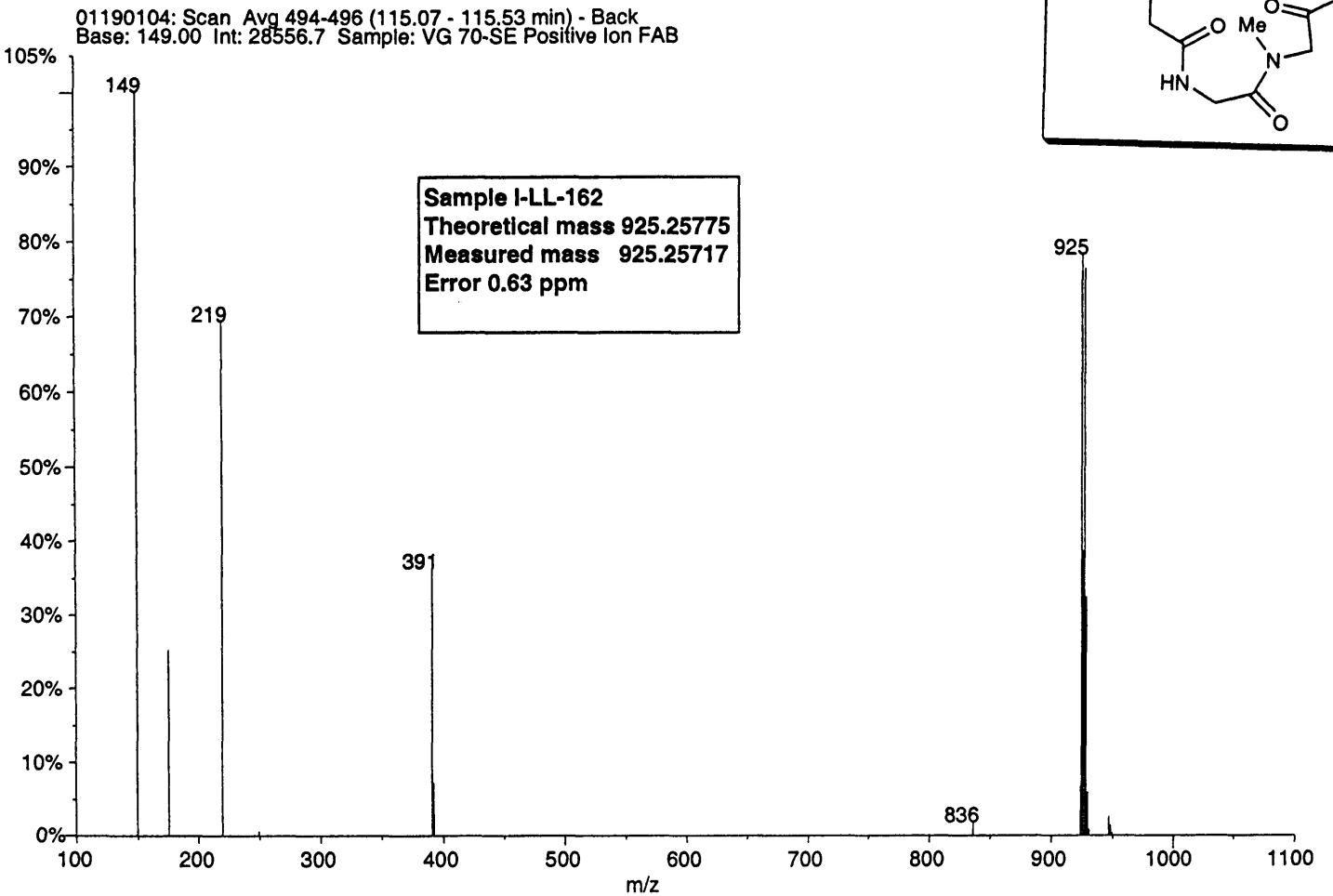


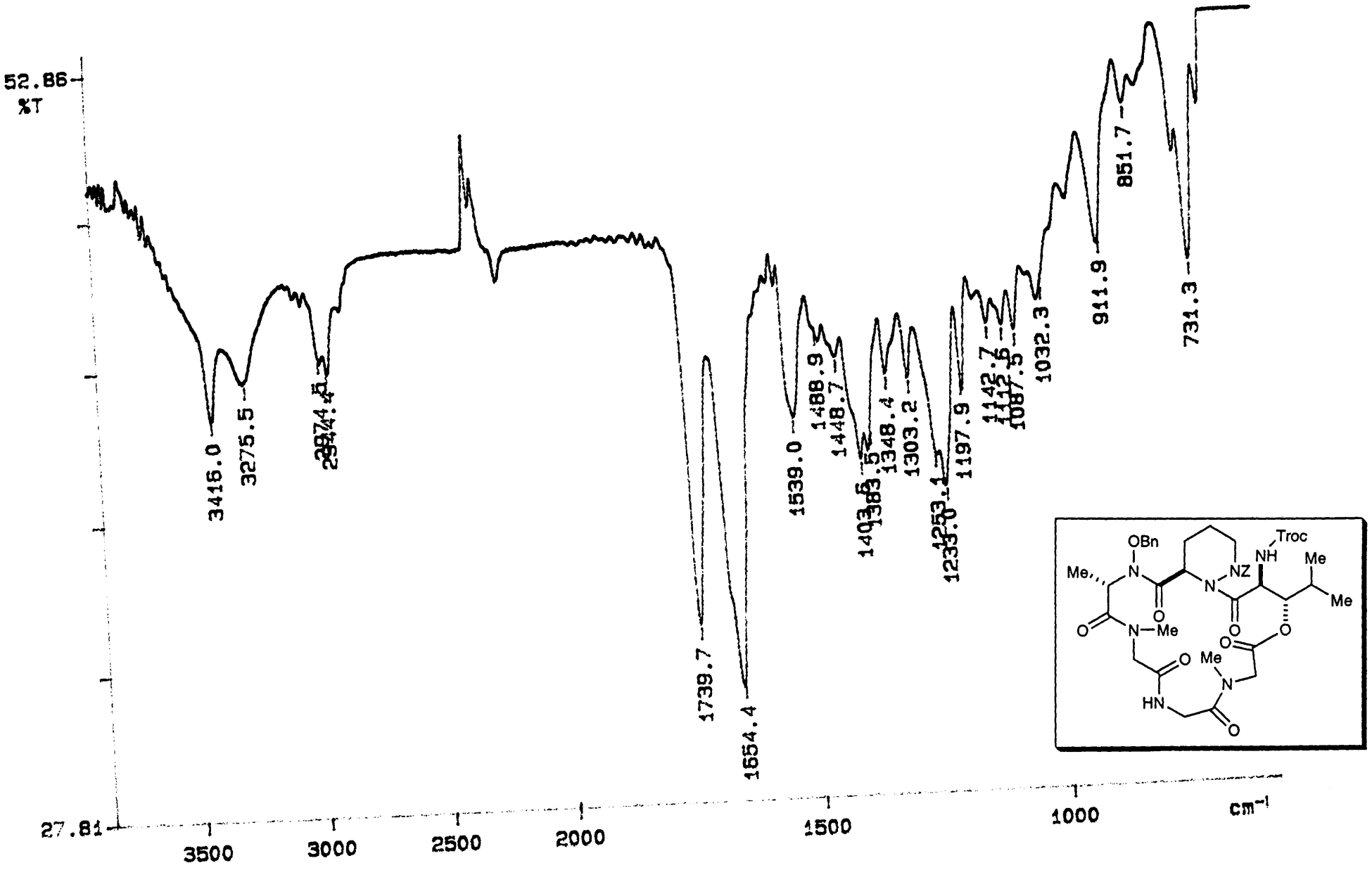
I-LL-162


OBn
Troc
NH
Me
Me
NZ
N
Me
N
Me
O
O
O
O
O
O
Me
N
HN
O

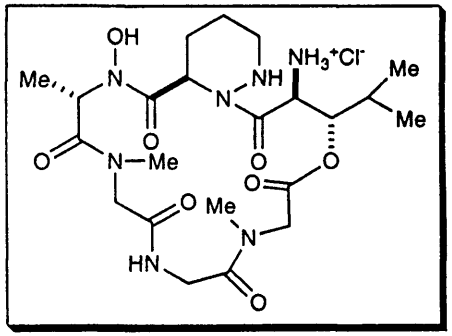
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


OBn
Troc
NH
Me
Me
Me
N
NZ
N
N
O
O
O
O
O
O
N
Me
O
Me
N
HN
O


01190104: Scan Avg 494-496 (115.07 - 115.53 min) - Back
Base: 149.00 Int: 28556.7 Sample: VG 70-SE Positive Ion FAB
Sample I-LL-162
Theoretical mass 925.25775
Measured mass 925.25717
Error 0.63 ppm
105%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
149
219
391
836
925
100
200
300
400
500
600
700
800
900
1000
1100
m/z

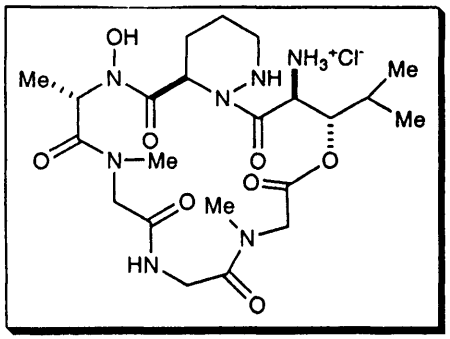

52.86
%T
27.81
3500
3000
2500
2000
1500
1000
cm⁻¹
3416.0
3275.5
2974.4
1739.7
1654.4
1539.0
1488.9
1448.7
1403.6
1383.5
1348.4
1303.2
1253.1
1233.0
1197.9
1142.7
1112.5
1087.5
1032.3
911.9
851.7
731.3
OBn
Troc
NH
Me
NZ
Me
Me
N
N
O
O
N—Me
O
O
O
O
Me
N
HN
O

I-LL-165


OH
N
Me
NH
NH3+Cl-
Me
Me
O
N
N
O
O
N–Me
O
O
O
Me
N
HN
O

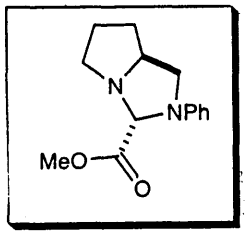
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

I-LL-165

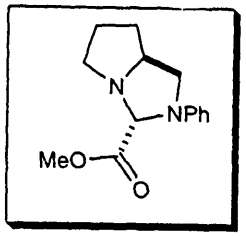

OH
N
Me
NH
NH3+Cl-
Me
Me
N
O
O
N–Me
O
O
O
O
Me
N
HN
O

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

III-LL-81

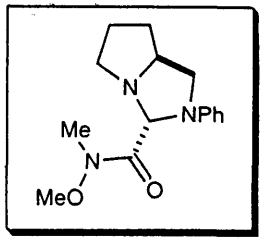


III-LL-81

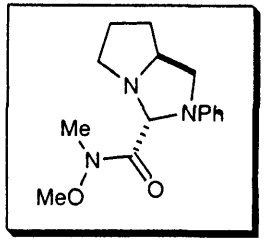

N
NPh
MeO
O

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

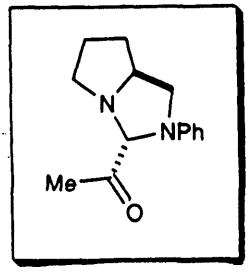
III-LL-82



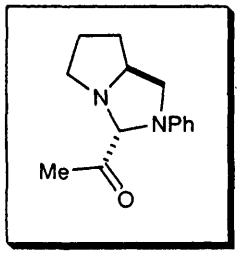
III-LL-82

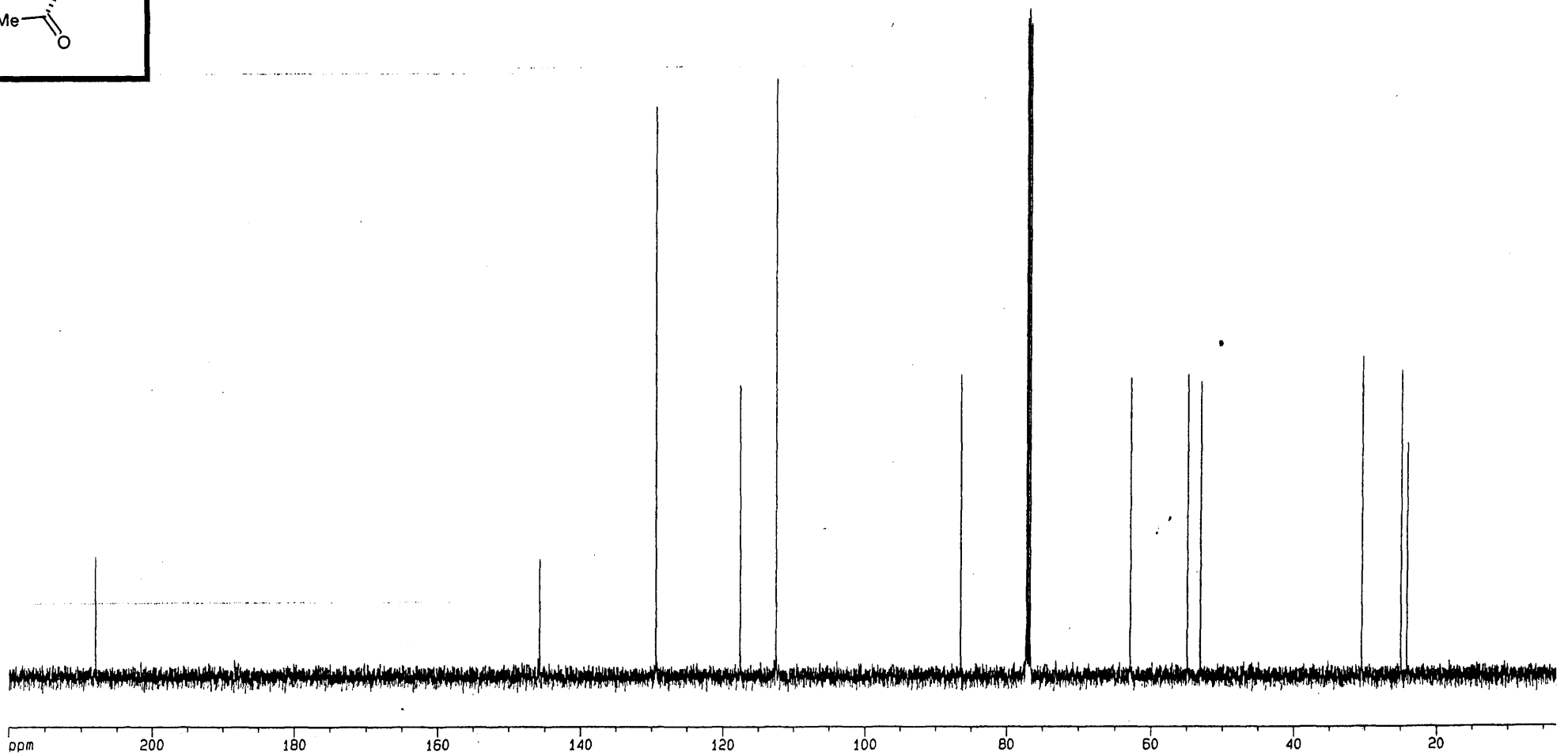

N
NPh
Me
N
MeO
O

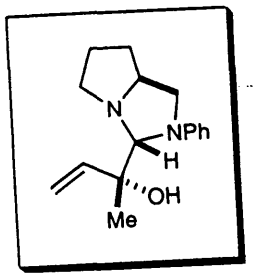
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


N
NPh
Me
O

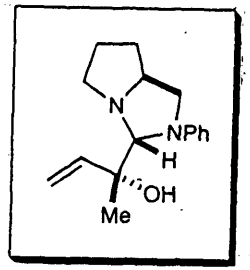
ppm
9
8
7
6
5
4
3
2
1
0


N
NPh
Me
O

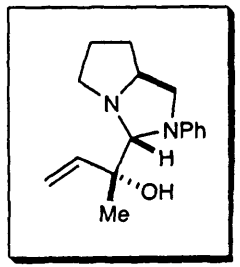

ppm
200
180
160
140
120
100
80
60
40
20


N
NPh
H
OH
Me

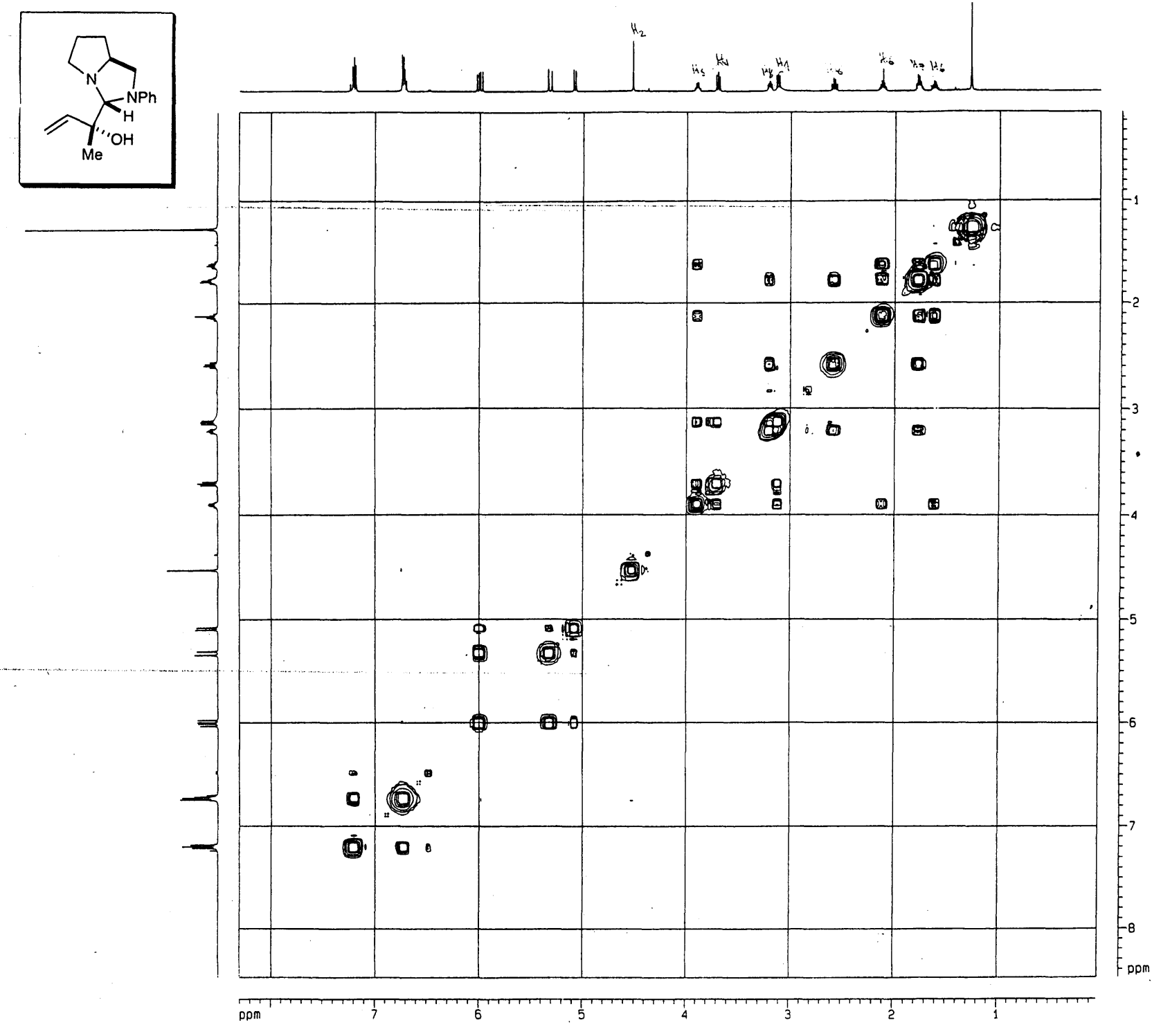
ppm
9
8
7
6
5
4
3
2
1
0

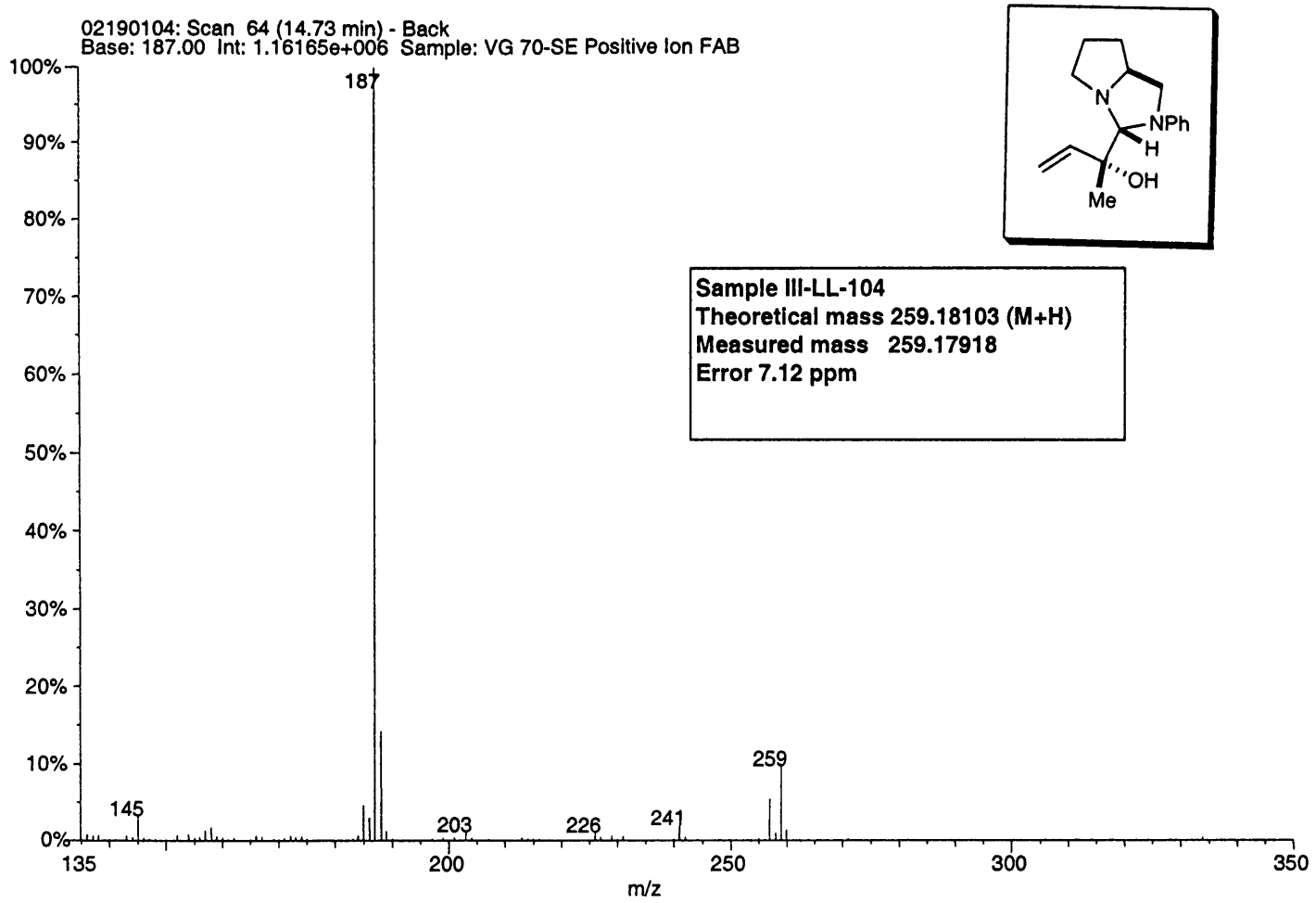

N
NPh
H
OH
Me

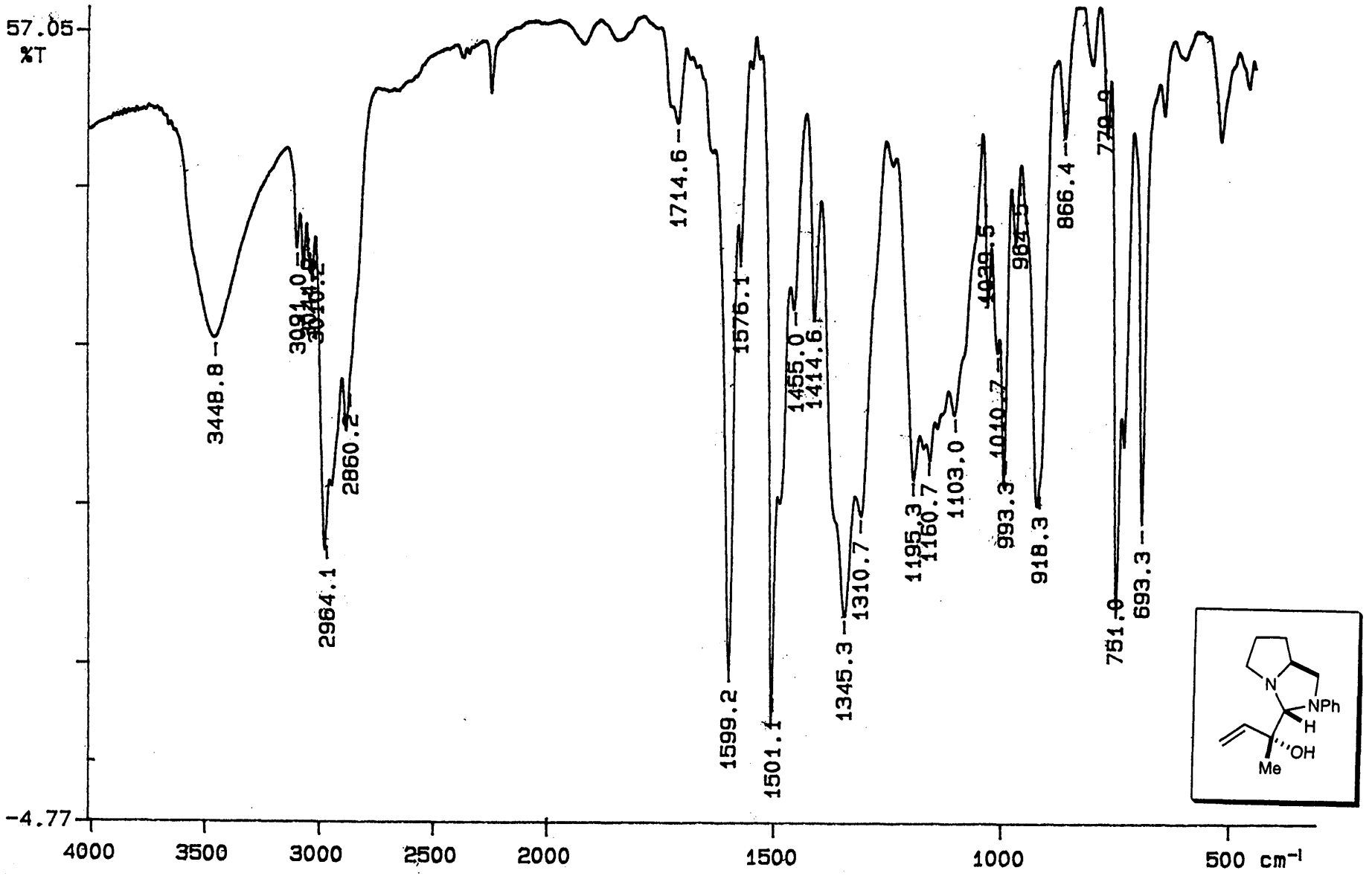
ppm
180
160
140
120
100
80
60
40
20
0


N
NPh
H
OH
Me

ppm
180
160
140
120
100
80
60
40
20
0

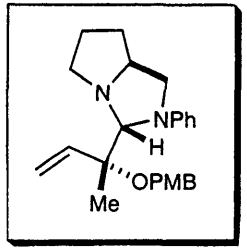

N
NPh
H
OH
Me
ppm
1
2
3
4
5
6
7
8
ppm
7
6
5
4
3
2
1


02190104: Scan 64 (14.73 min) - Back
Base: 187.00 Int: 1.16165e+006 Sample: VG 70-SE Positive Ion FAB
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
187
145
203
226
241
259
135
200
250
300
350
m/z
N
NPh
H
OH
Me
Sample III-LL-104
Theoretical mass 259.18103 (M+H)
Measured mass 259.17918
Error 7.12 ppm

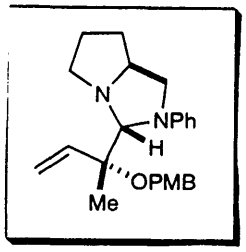

57.05
%T
-4.77
4000
3500
3000
2500
2000
1500
1000
500 cm-1
3448.8
2964.1
2860.2
1714.6
1599.2
1576.1
1501.1
1455.0
1414.6
1345.3
1310.7
1195.3
1160.7
1103.0
1039.5
1010.7
993.3
964.1
918.3
866.4
751.0
693.3
N
NPh
H
OH
Me

III-CC-104

III-LL-105

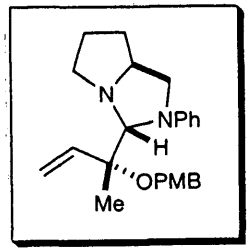


III-LL-105


N
NPh
H
OPMB
Me

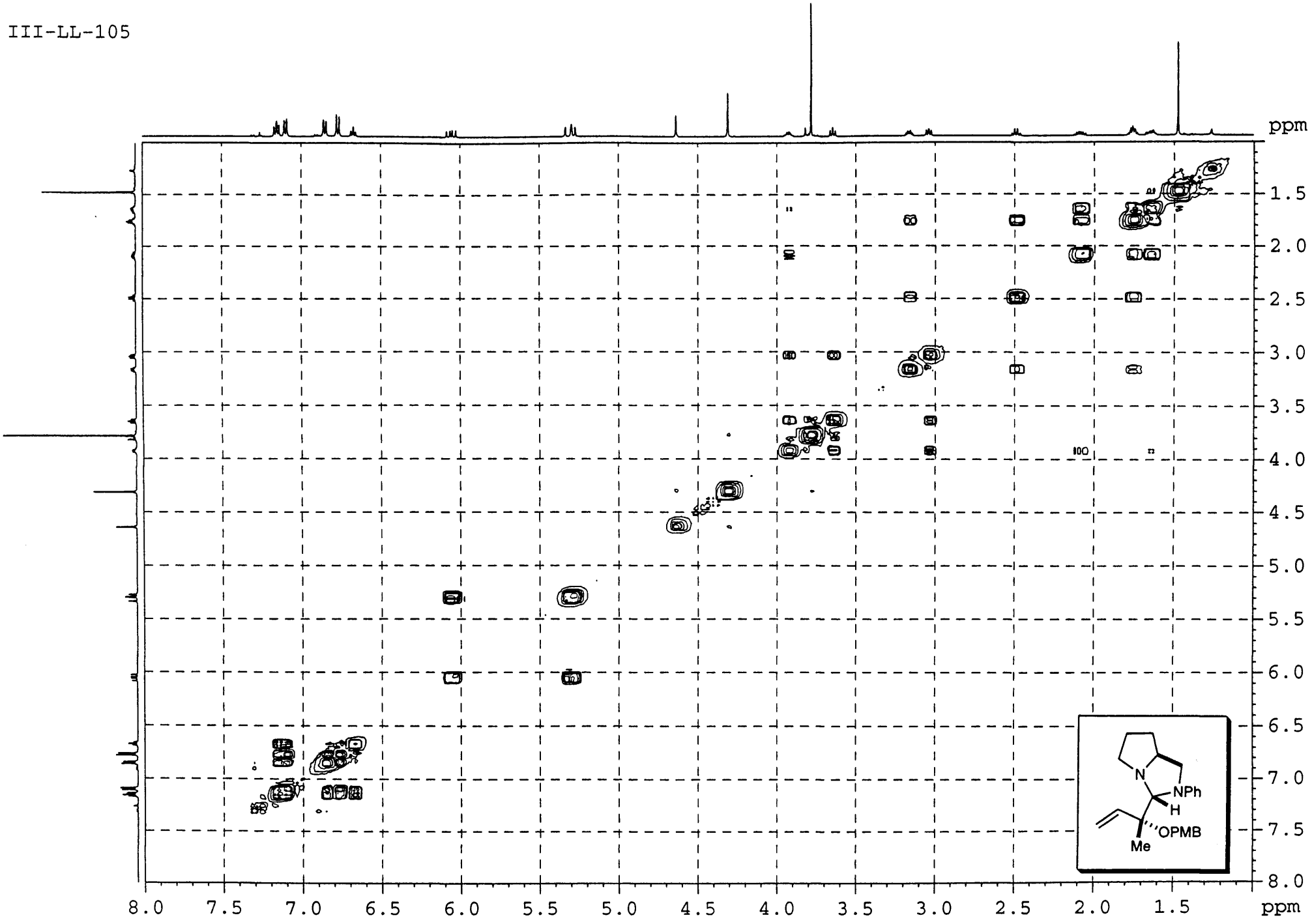
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

III-LL_105

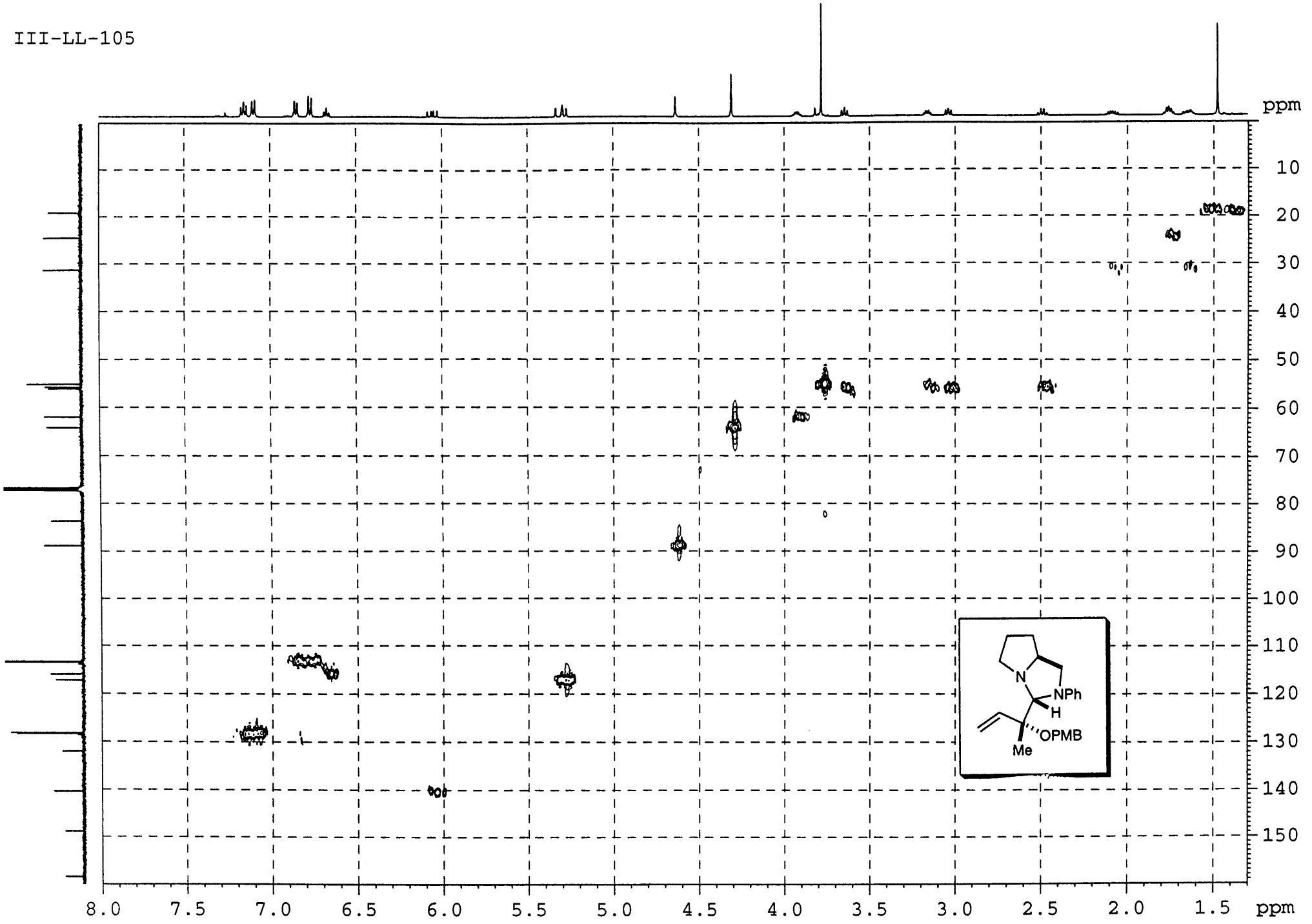

N
NPh
H
OPMB
Me

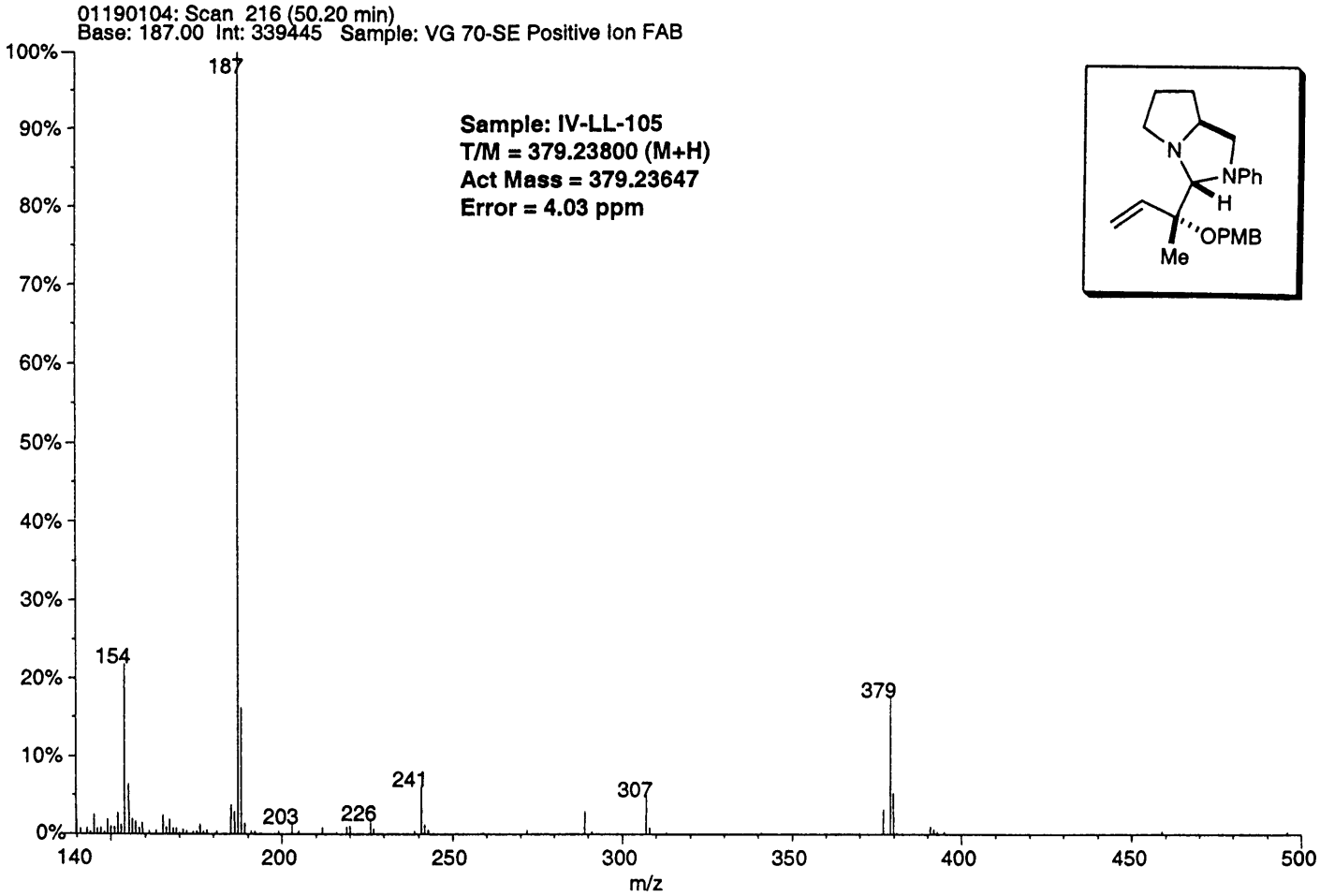
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

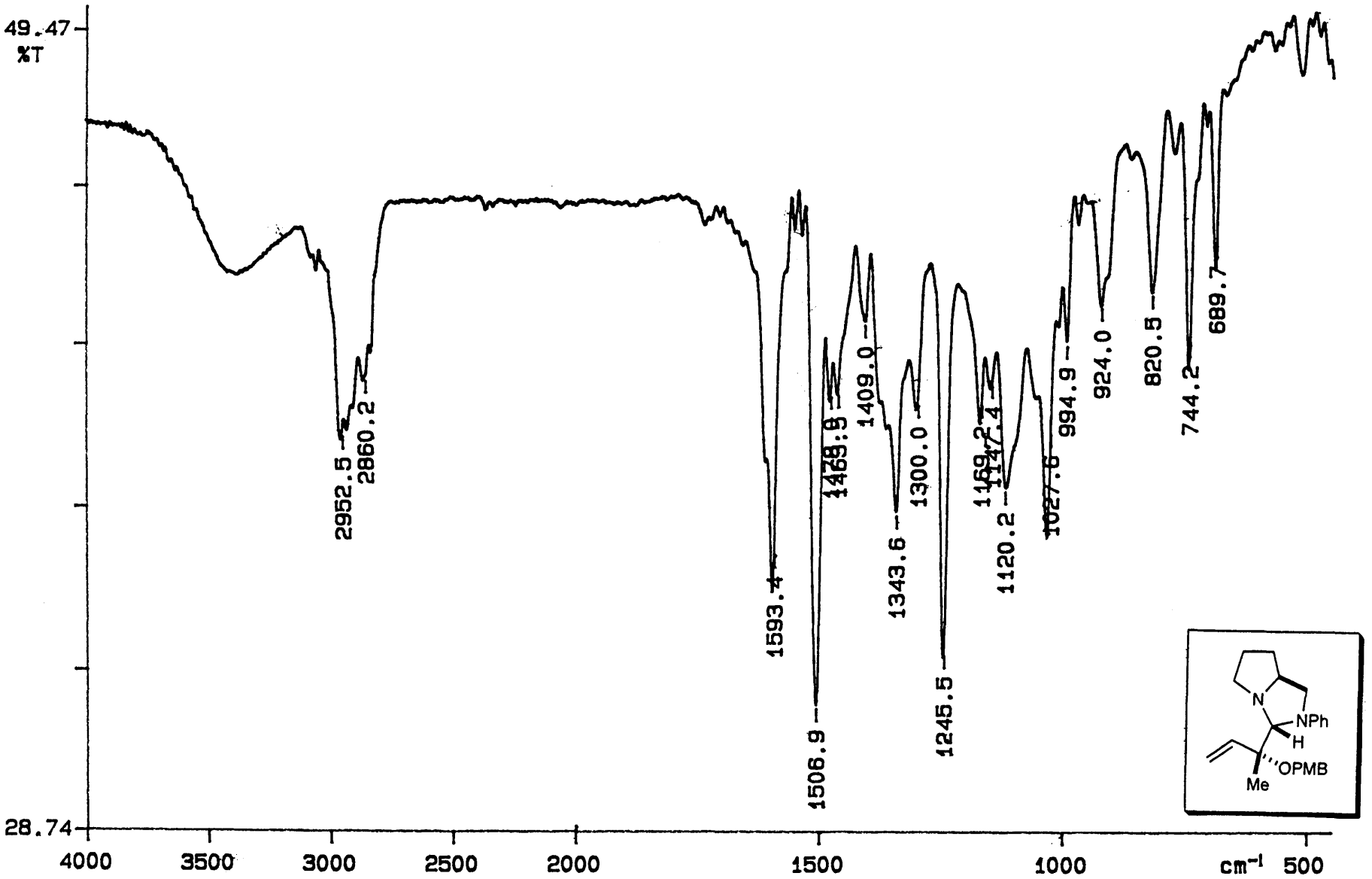
III-LL-105


ppm
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
N
NPh
H
OPMB
Me

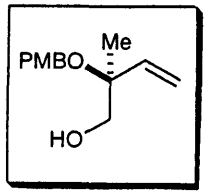
III-LL-105


ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
N
NPh
H
OPMB
Me

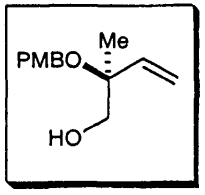

01190104: Scan 216 (50.20 min)
Base: 187.00 Int: 339445 Sample: VG 70-SE Positive Ion FAB
Sample: IV-LL-105
T/M = 379.23800 (M+H)
Act Mass = 379.23647
Error = 4.03 ppm
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
187
154
203
226
241
307
379
140
200
250
300
350
400
450
500
m/z
N
NPh
H
OPMB
Me


49.47
%T
28.74
2952.5
2860.2
1593.4
1506.9
1469.9
1409.0
1343.6
1300.0
1245.5
1169.2
1147.4
1120.2
1027.6
994.9
924.0
820.5
744.2
689.7
4000
3500
3000
2500
2000
1500
1000
cm-1
500
N
NPh
H
OPMB
Me

IV-LL-36

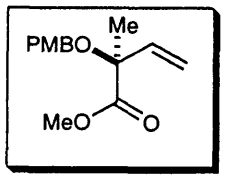


IV-LL-36

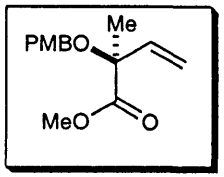

Me
PMBO
HO

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

IV-LL-161

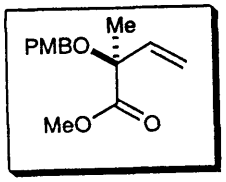


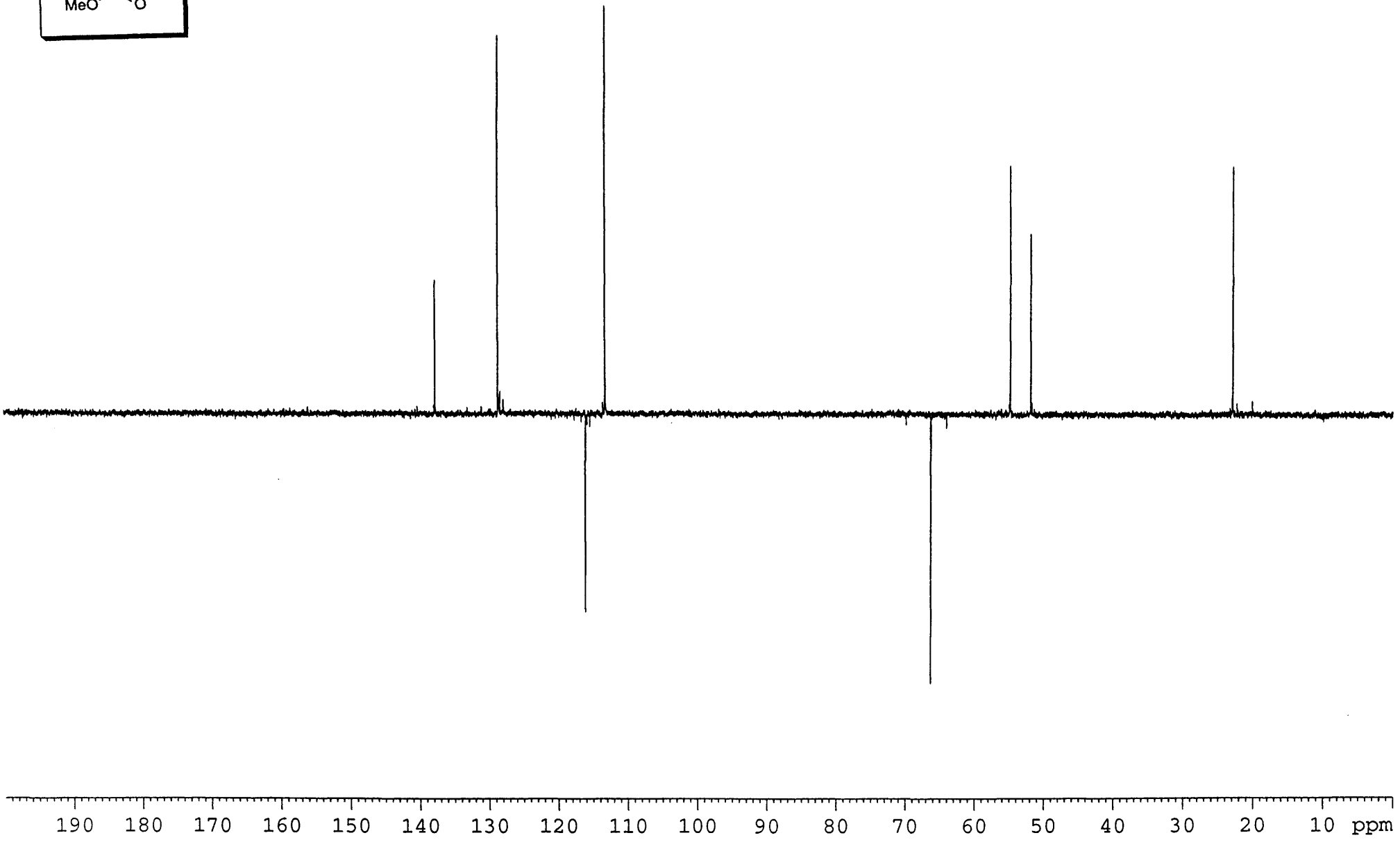
IV-LL-161


Me
PMBO
MeO
O

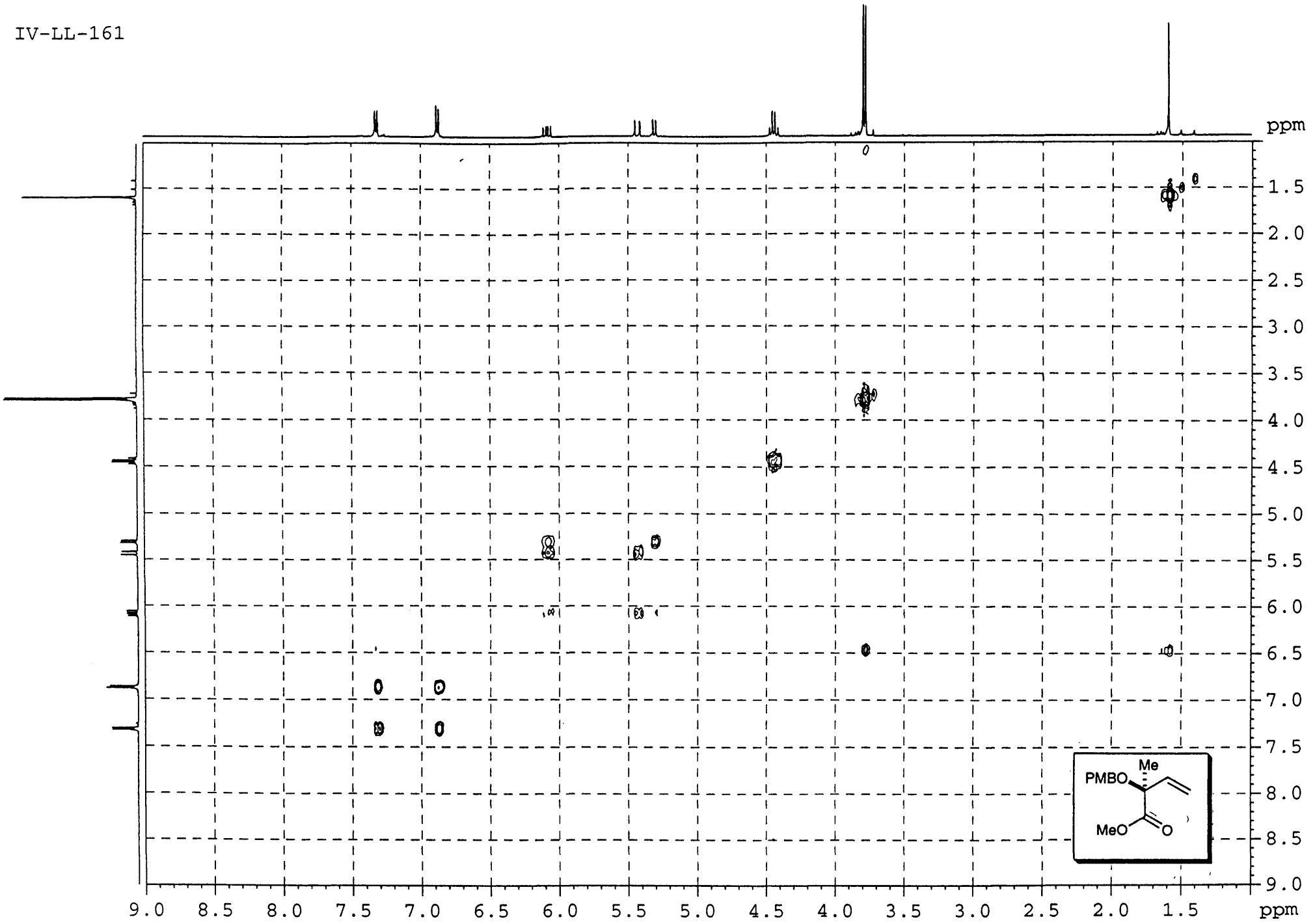
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

IV-LL-161

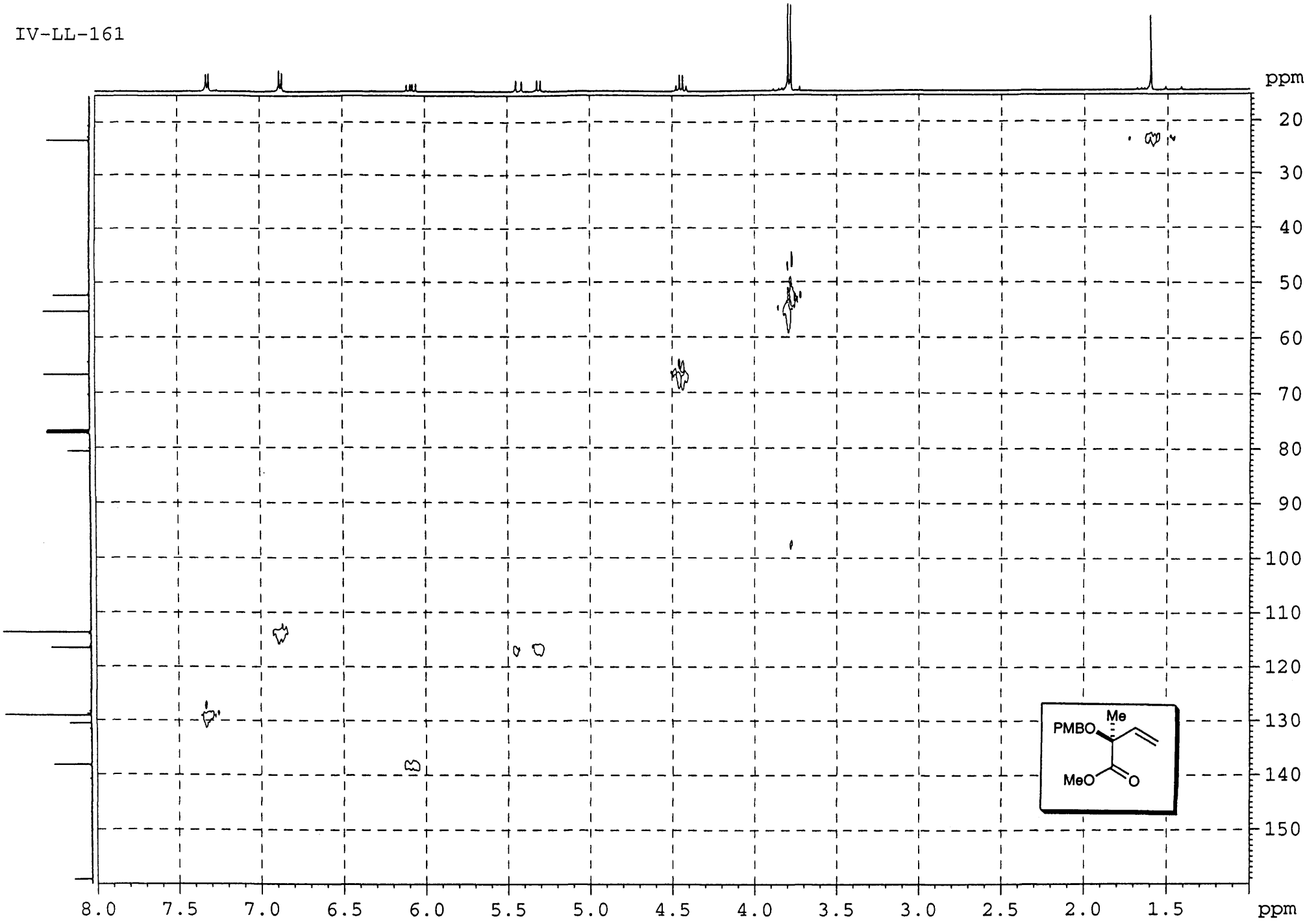

Me
PMBO
MeO
O

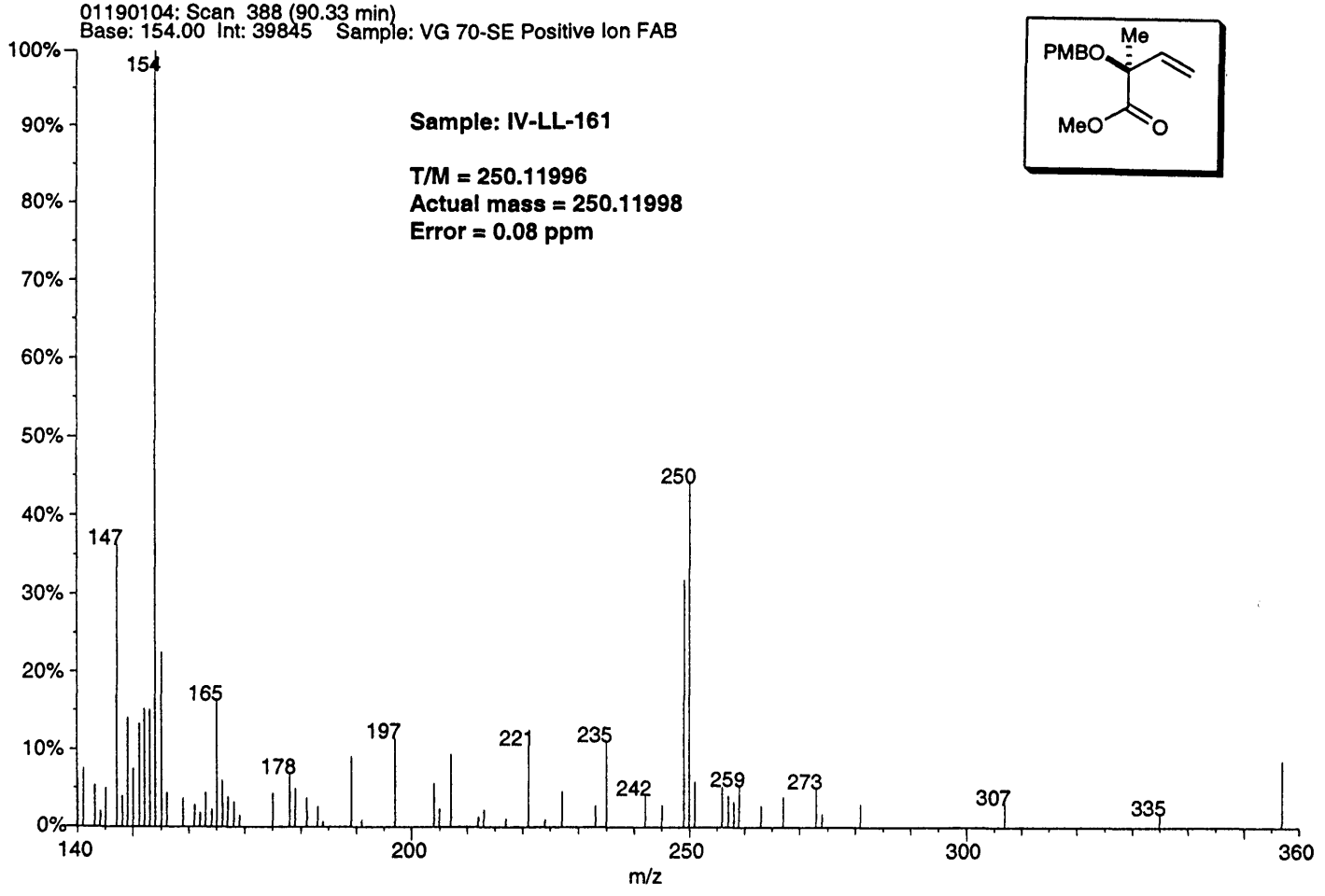

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

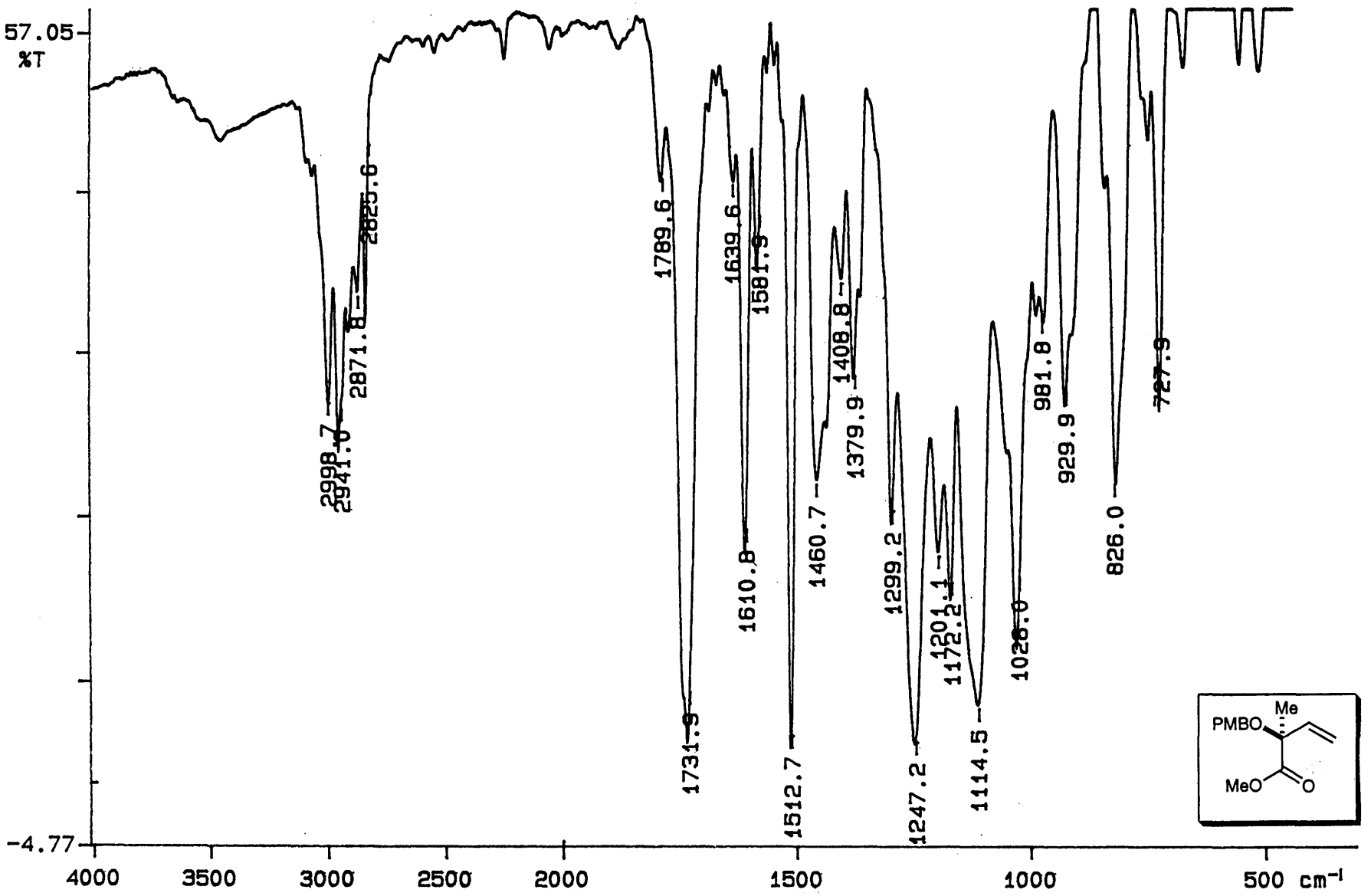
IV-LL-161


ppm
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 ppm
Me
PMBO
MeO
O

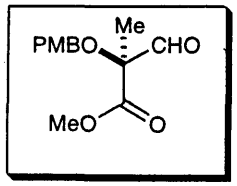
IV-LL-161


ppm
20
30
40
50
60
70
80
90
100
110
120
130
140
150
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
PMBO
Me
MeO
O

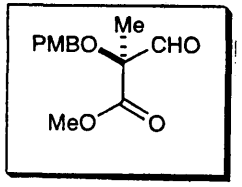

01190104: Scan 388 (90.33 min)
Base: 154.00 Int: 39845 Sample: VG 70-SE Positive Ion FAB
Sample: IV-LL-161
T/M = 250.11996
Actual mass = 250.11998
Error = 0.08 ppm
PMBO
Me
MeO
O
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
154
147
165
178
197
221
235
242
250
259
273
307
335
140
200
250
300
360
m/z


57.05
%T
-4.77
4000
3500
3000
2500
2000
1500
1000
500 cm-1
2998.7
2941.0
2871.8
2825.6
1789.6
1731.9
1639.6
1610.8
1581.5
1512.7
1460.7
1408.8
1379.9
1299.2
1247.2
1201.1
1172.2
1114.5
1028.0
981.8
929.9
826.0
727.9
PMBO
Me
MeO
O

IV-LL-179

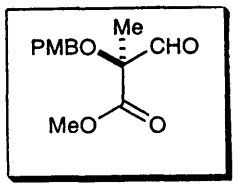


IV-LL-179


Me
PMBO
CHO
MeO
O

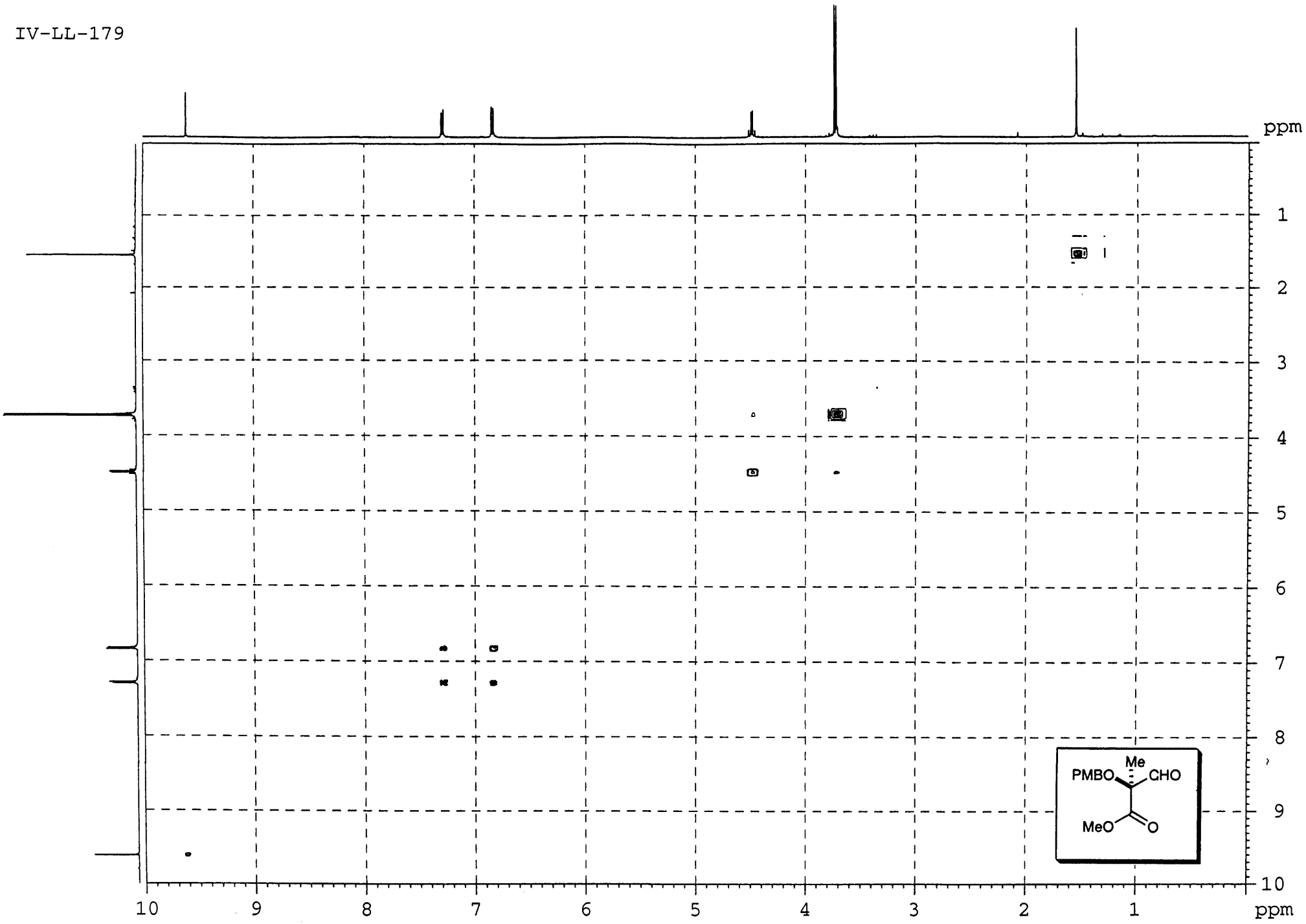
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

IV-LL-179

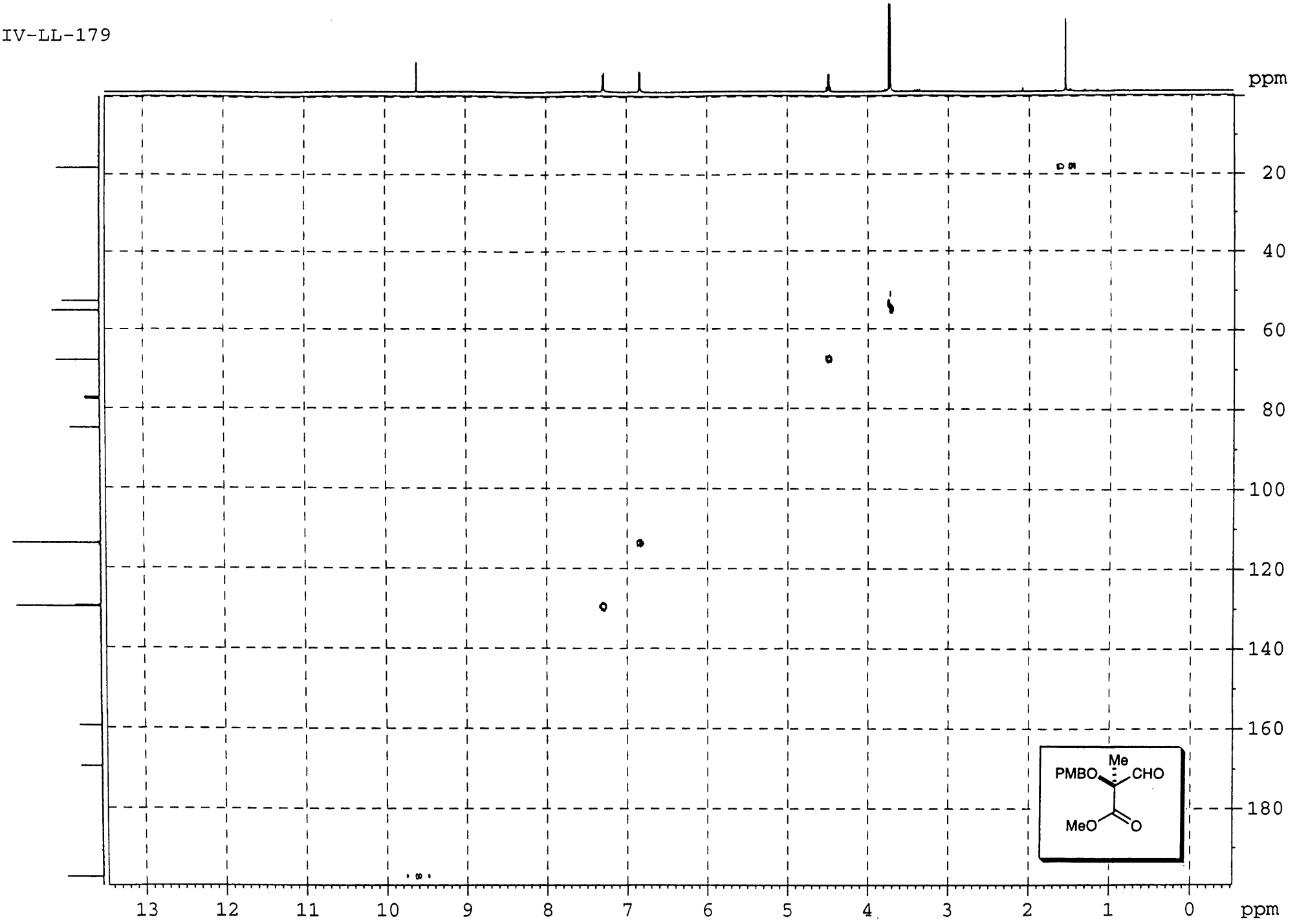

Me
PMBO
CHO
MeO
O

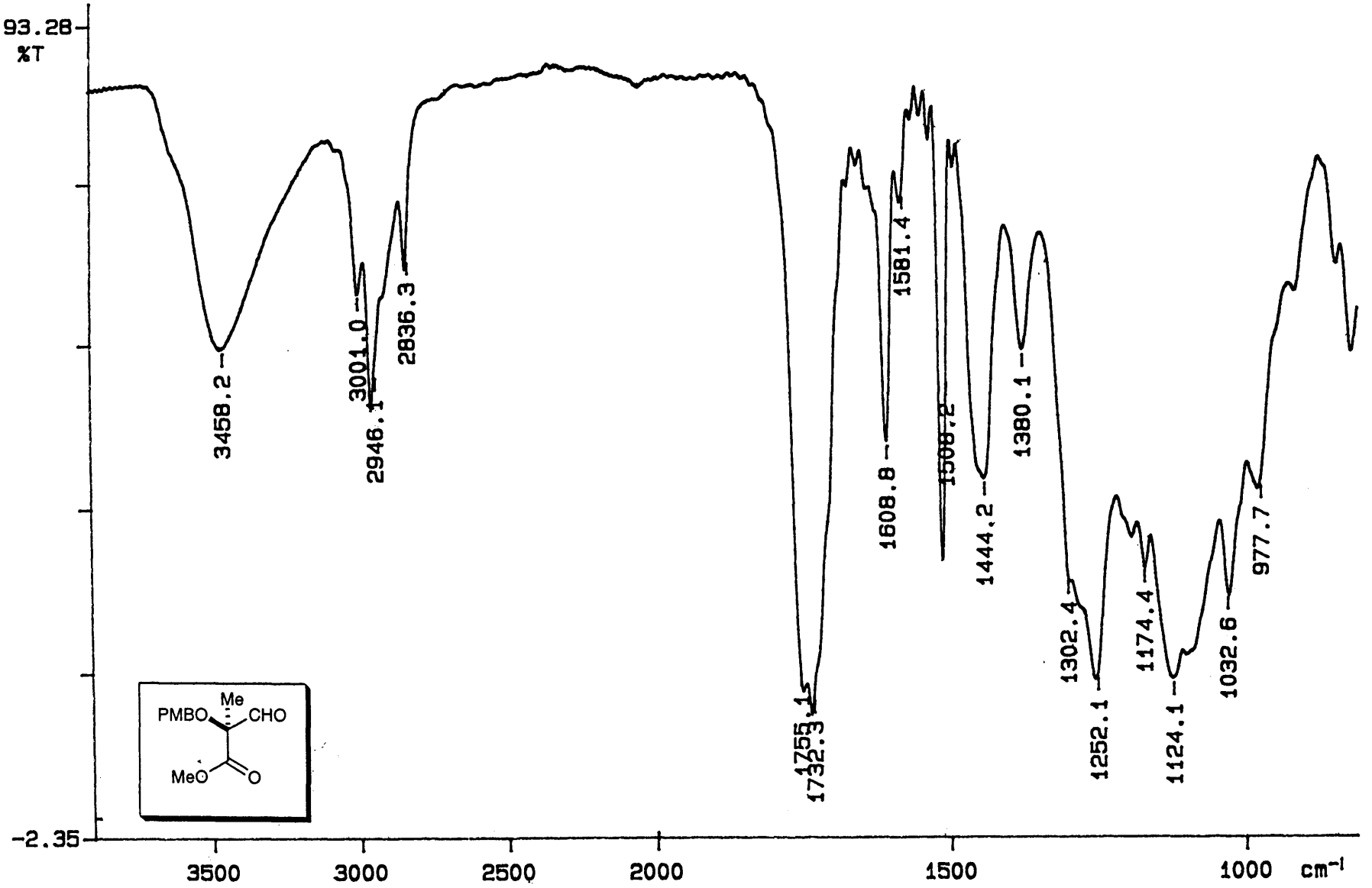
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

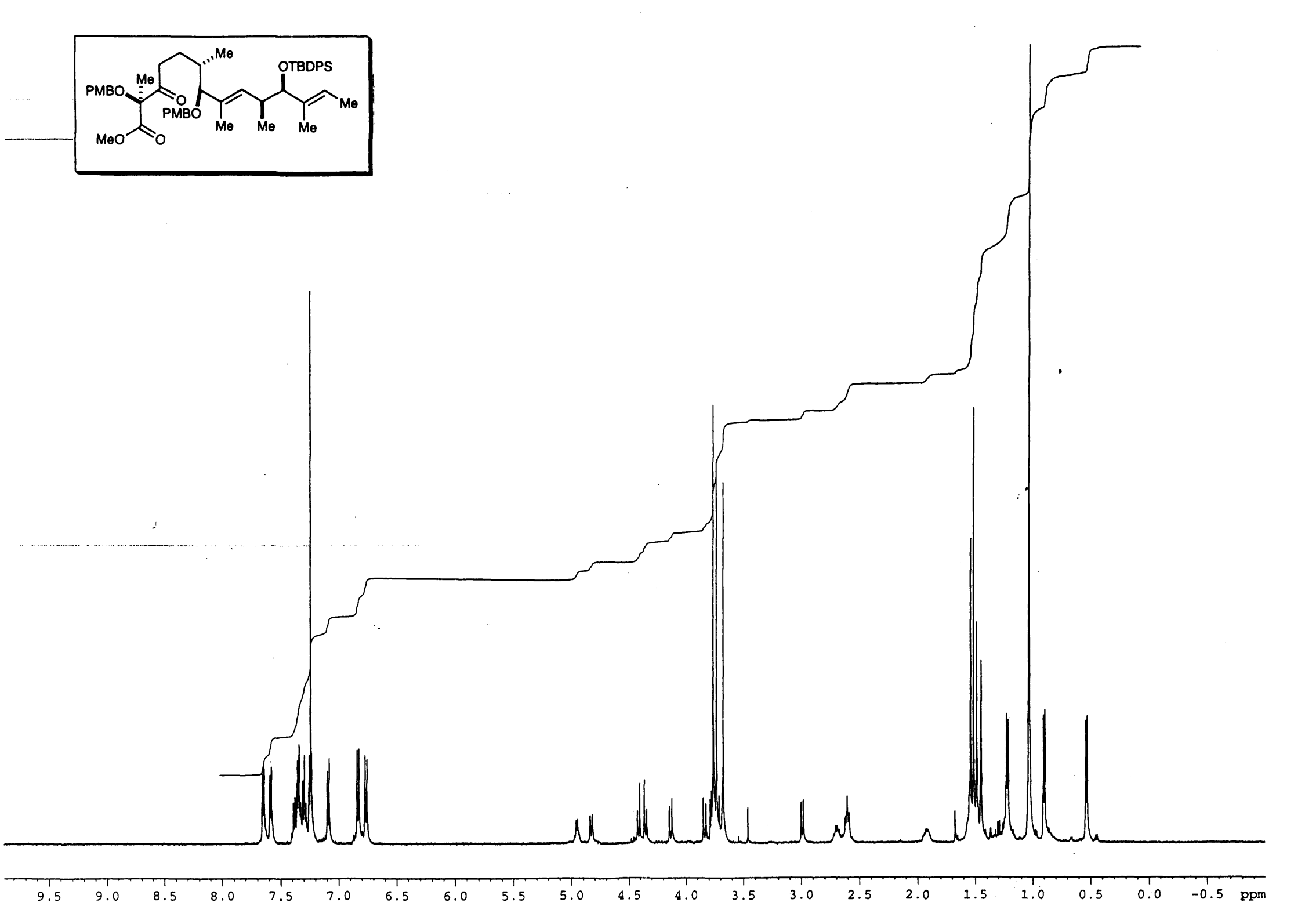
IV-LL-179

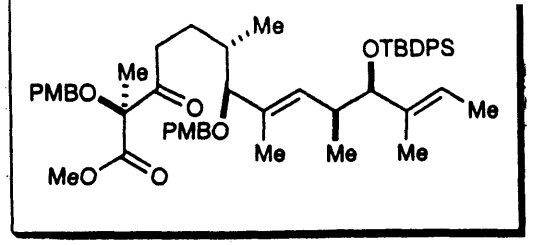


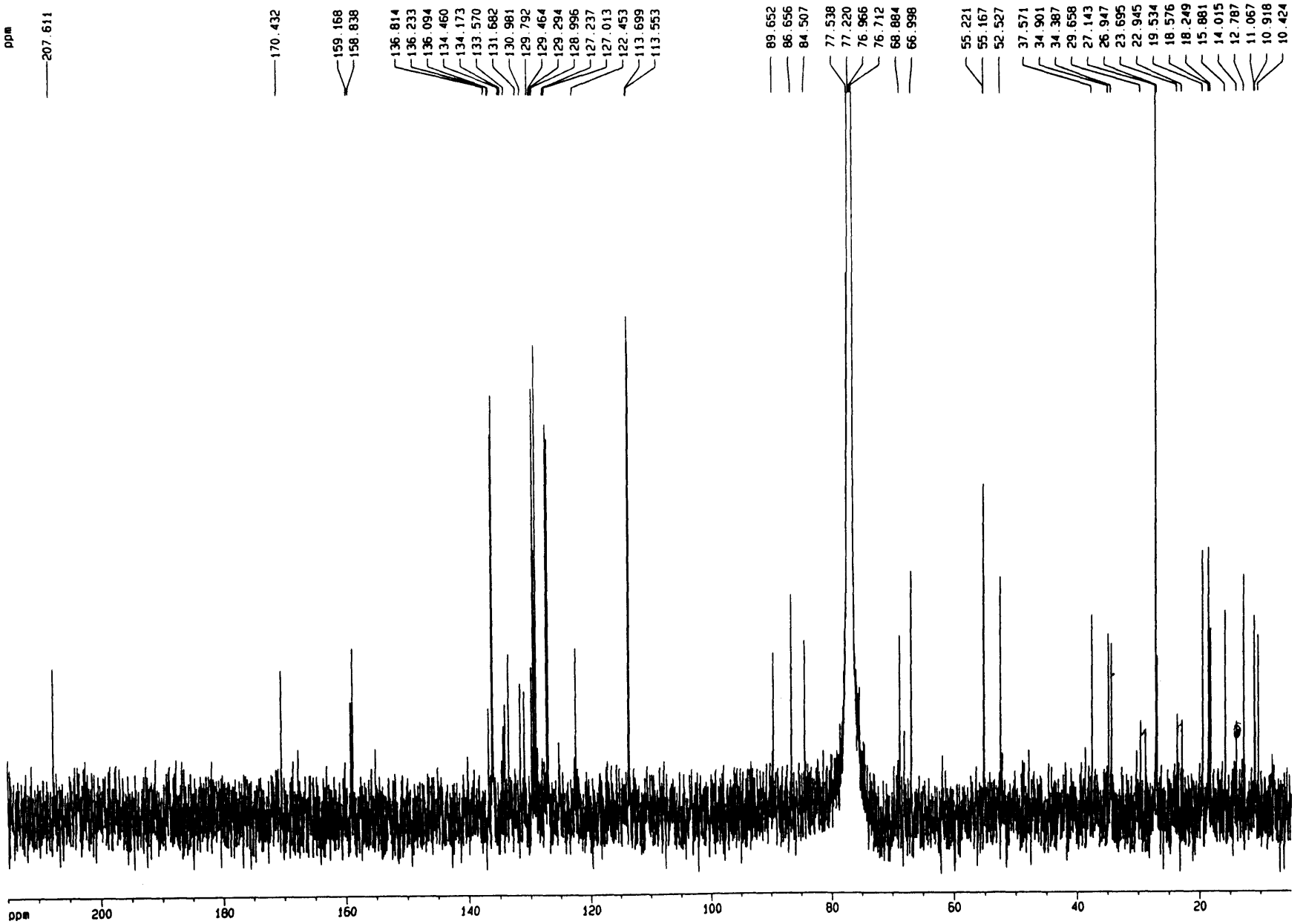
IV-LL-179


ppm
20
40
60
80
100
120
140
160
180
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
Me
PMBO
CHO
MeO
O


93.28
%T
3458.2
3001.0
2946.1
2836.3
1755.1
1732.3
1608.8
1581.4
1508.2
1444.2
1380.1
1302.4
1252.1
1174.4
1124.1
1032.6
977.7
-2.35
3500
3000
2500
2000
1500
1000
cm-1
PMBO
Me
CHO
MeO
O


Me
OTBDPS
Me
PMBO
O
PMBO
Me
Me
Me
Me
MeO
O
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
ppm





Current Data Parameters

NAME	IV-LL-86
EXPNO	10
PROCNO	1

F2 - Acquisition Parameters

Date_	20030929
Time	7.03
INSTRUM	drx500
PROBHD	5 mm Multinu
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	20480
DS	4
SWH	31446.541 Hz
FIDRES	0.479836 Hz
AQ	1.0420724 sec
RG	11585.2
DW	15.900 usec
DE	6.00 usec
TE	300.0 K
D1	2.00000000 sec
d11	0.03000000 sec
d12	0.00002000 sec

---------- CHANNEL f1 ----------

NUC1	13C
P1	4.75 usec
PL1	0.00 dB
SF01	125.7715719 MHz

---------- CHANNEL f2 ----------

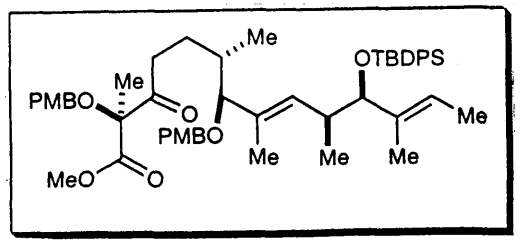
CPDPRG2	waltz16
NUC2	1H
PCPD2	95.00 usec
PL2	0.00 dB
PL12	19.00 dB
PL13	19.00 dB
SF02	500.1320005 MHz

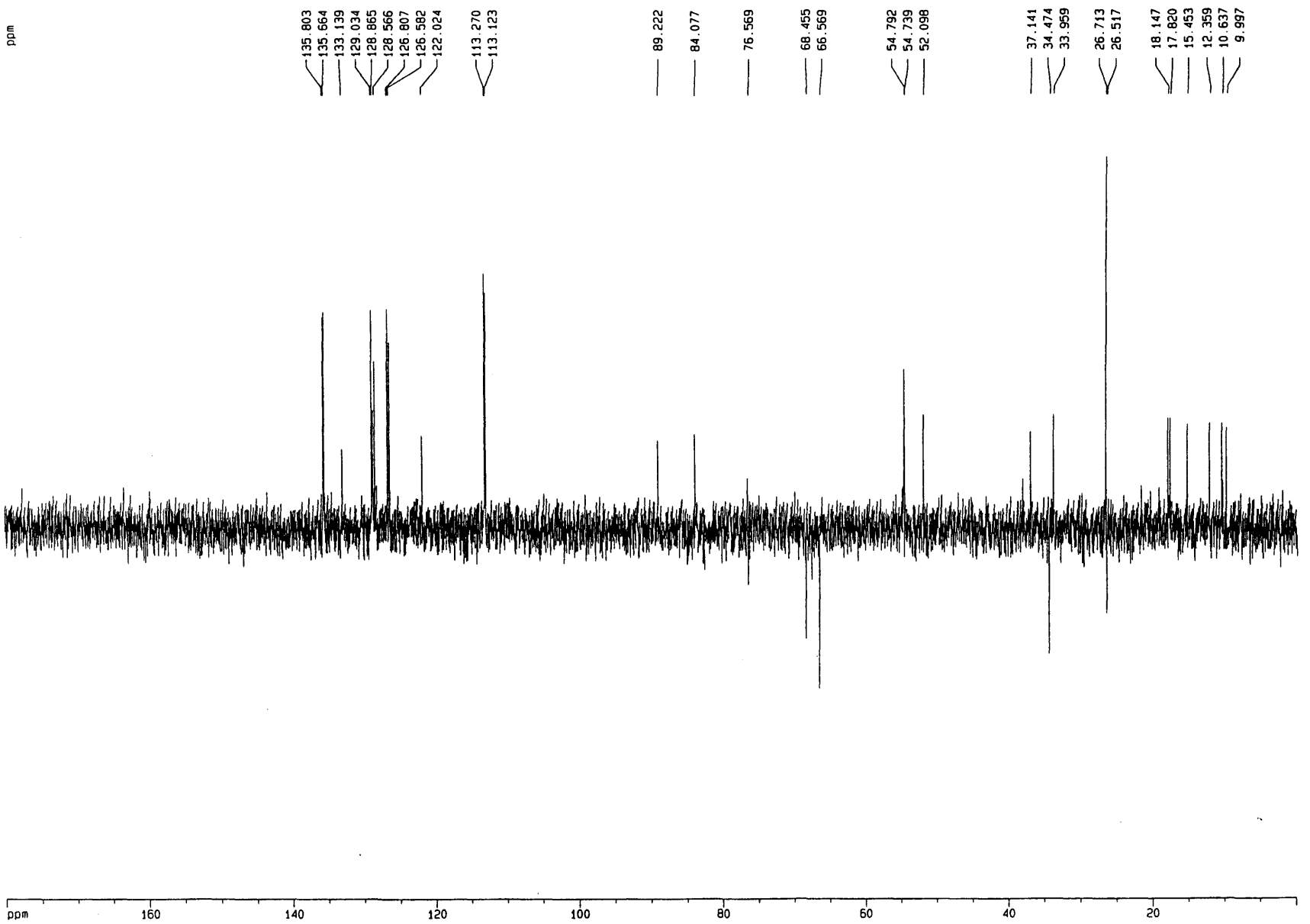
F2 - Processing parameters

SI	131072
SF	125.7577965 MHz
WDW	EM
SSB	0
LB	2.00 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	32.00 cm
F1P	215.000 ppm
F1	27037.93 Hz
F2P	5.000 ppm
F2	628.79 Hz
PPMCM	6.56250 ppm/cm
HZCM	825.28546 Hz/cm





Current Data Parameters

NAME	IV-LL-86
EXPNO	6
PROCNO	1

F2 - Acquisition Parameters

Date_	20030927
Time	4.52
INSTRUM	drx500
PROBHD	5 mm Multinu
PULPROG	dept135
TD	65536
SOLVENT	CDCl3
NS	3584
DS	4
SWH	31446.541 Hz
FIDRES	0.479836 Hz
AQ	1.0420724 sec
RG	6502
DW	15.900 usec
DE	6.00 usec
TE	300.0 K
CNST2	145.0000000
D1	2.00000000 sec
d2	0.00344828 sec
d12	0.00002000 sec
DELTA	0.00000605 sec

========== CHANNEL f1 ==========

NUC1	13C
P1	4.75 usec
p2	9.50 usec
PL1	0.00 dB
SFO1	125.7715719 MHz

========== CHANNEL f2 ==========

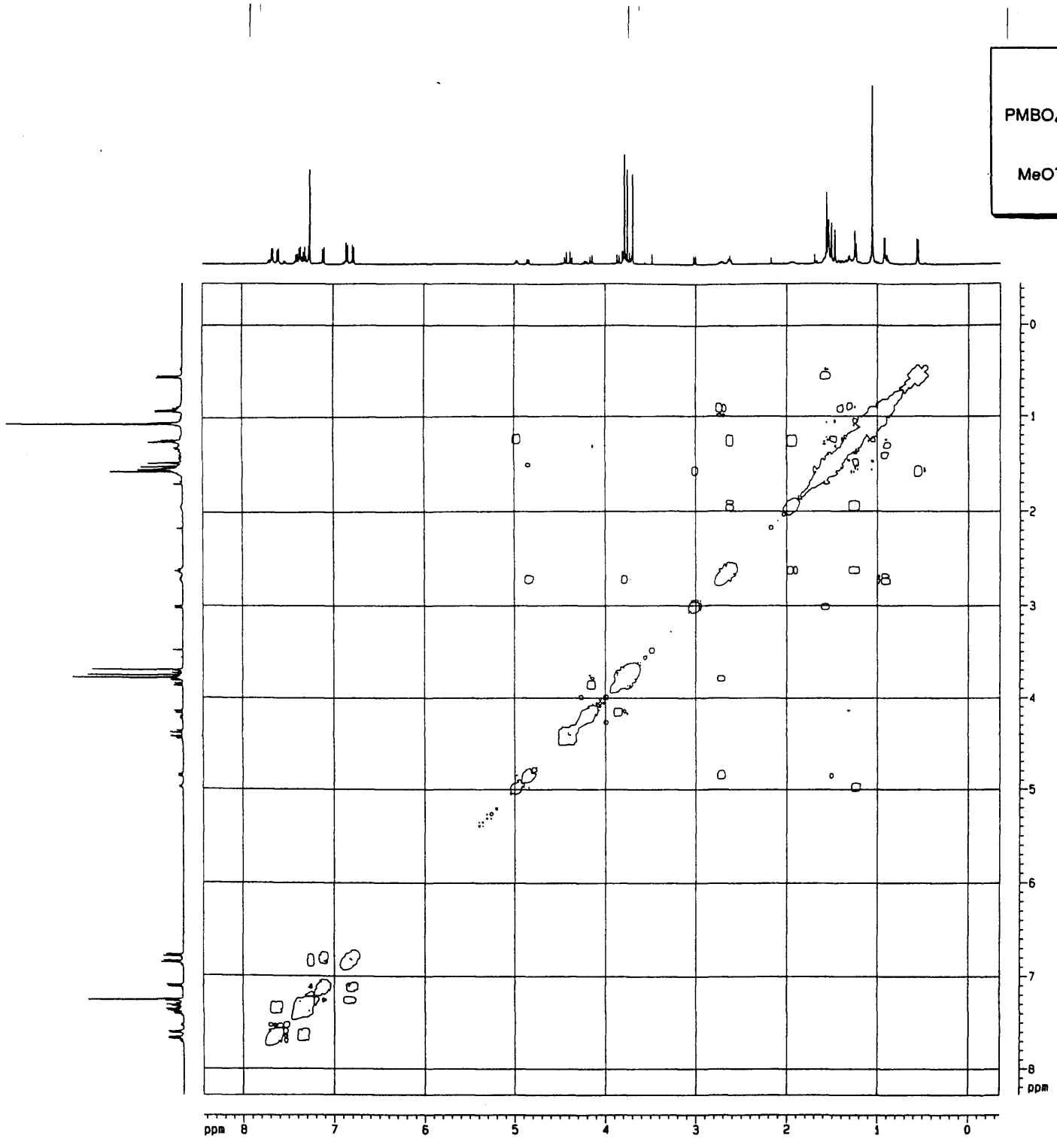
CPDPRG2	waltz16
NUC2	1H
P3	11.50 usec
p4	23.00 usec
PCPD2	95.00 usec
PL2	0.00 dB
PL12	19.00 dB
SFO2	500.1320005 MHz

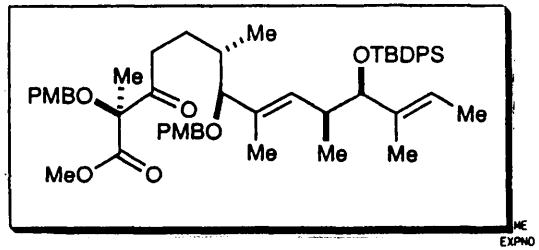
F2 - Processing parameters

SI	131072
SF	125.7578505 MHz
WDW	EM
SSB	0
LB	2.00 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	32.00 cm
F1P	180.000 ppm
F1	22636.41 Hz
F2P	0.000 ppm
F2	0.00 Hz
PPMCM	5.62500 ppm/cm
HZCM	707.38794 Hz/cm





Current Data Parameters

NAME	IV-LL-86
EXPNO	4
PROCNO	1

F2 - Acquisition Parameters

Date_	20030926
Time	11.45
INSTRUM	drx500
PROBHD	5 mm Multinu
PULPROG	cosygs
TD	2048
SOLVENT	CDCl3
NS	25
DS	0
SWH	7246.377 Hz
FIDRES	3.538270 Hz
AQ	0.1413620 sec
RG	4597.6
DW	69.000 usec
DE	6.00 usec
TE	300.0 K
d0	0.00000300 sec
D1	1.00000000 sec
d13	0.00000300 sec
D16	0.00010000 sec
IN0	0.00013800 sec

CHANNEL f1

NUC1	1H
P0	11.50 usec
P1	11.50 usec
PL1	0.00 dB
SFO1	500.1332574 MHz

GRADIENT CHANNEL

P16	1000.00 usec

F1 - Acquisition parameters

ND0	1
TD	206
SFO1	500.1333 MHz
FIDRES	35.176586 Hz
SW	14.489 ppm

F2 - Processing parameters

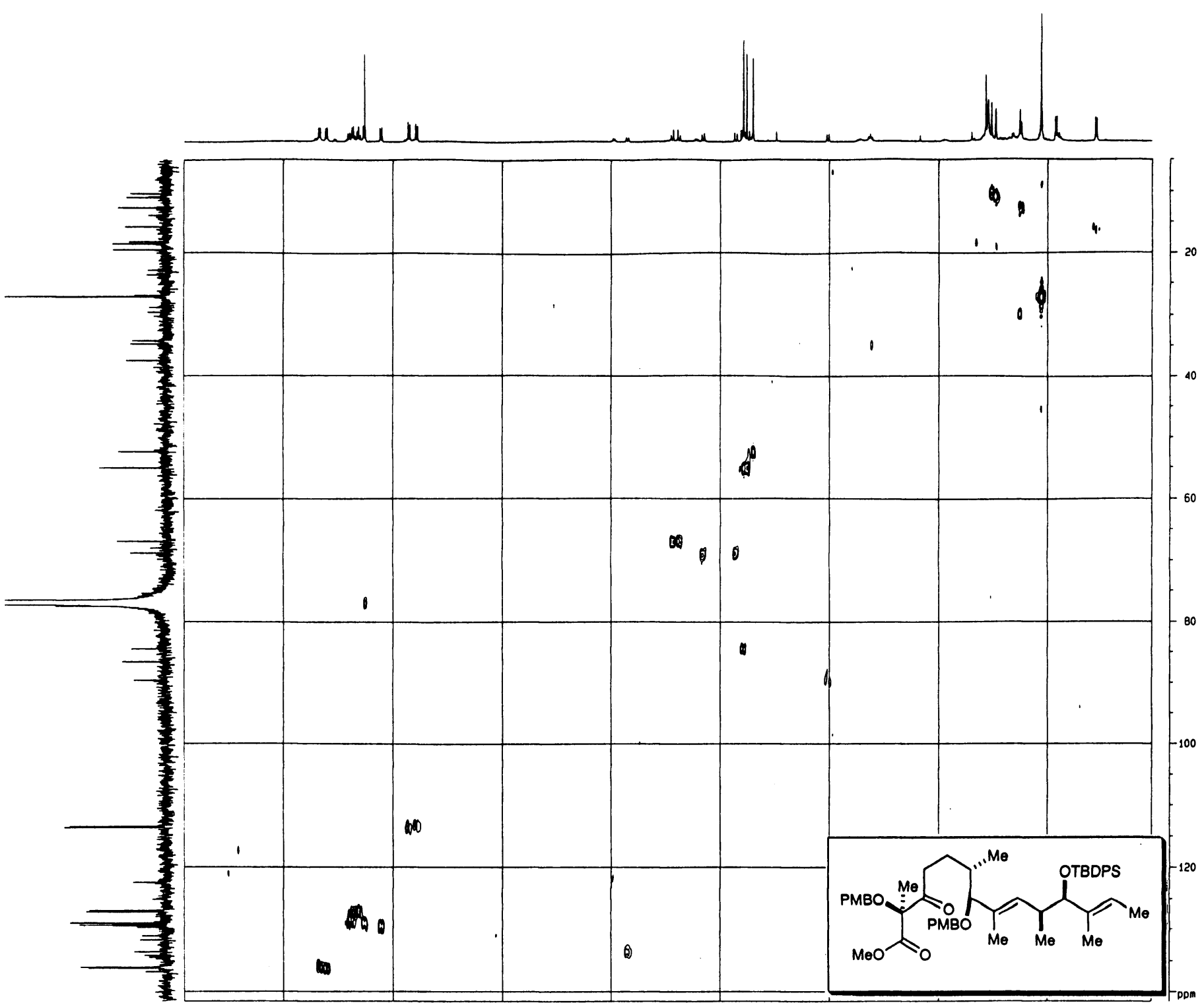
SI	1024
SF	500.1300140 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.40

F1 - Processing parameters

SI	1024
MC2	QF
SF	500.1300140 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	20.00 cm
CX1	20.00 cm
F2PLO	8.438 ppm
F2LO	4220.04 Hz
F2PHI	-0.349 ppm
F2HI	-174.49 Hz
F1PLO	8.282 ppm
F1LO	4142.20 Hz
F1PHI	-0.462 ppm
F1HI	-231.10 Hz
F2PPMCM	0.43934 ppm/cm
F2HZCM	219.72655 Hz/cm
F1PPMCM	0.43722 ppm/cm
F1HZCM	218.66507 Hz/cm



Current Data Parameters

NAME	IV-LL-86
EXPNO	7
PROCNO	1

F2 - Acquisition Parameters

Date_	20030927
Time	4.55
INSTRUM	drx500
PROBHD	5 mm Multinu
PULPROG	inv4gs
TD	2048
SOLVENT	CDCl3
NS	100
DS	8
SWH	7002.801 Hz
FIDRES	3.419337 Hz
AQ	0.1462772 sec
RG	16384
DW	71.400 usec
DE	6.00 usec
TE	300.0 K
CNST2	145.0000000
d0	0.00000300 sec
D1	1.44101703 sec
d2	0.00344828 sec
d12	0.00002000 sec
d13	0.00000300 sec
D16	0.00010000 sec
d20	0.00242528 sec
IN0	0.00001988 sec

CHANNEL f1

NUC1	1H
P1	11.50 usec
p2	23.00 usec
PL1	0.00 dB
SFO1	500.1332508 MHz

CHANNEL f2

CPDPRG2	garp
NUC2	13C
P3	4.80 usec
PCPD2	70.00 usec
PL2	0.00 dB
PL12	25.00 dB
SFO2	125.7703148 MHz

GRADIENT CHANNEL

P16	1000.00 usec

F1 - Acquisition parameters

NDO	2
TD	128
SFO1	125.7703 MHz
FIDRES	196.540878 Hz
SW	200.025 ppm

F2 - Processing parameters

SI	2048
SF	500.1300140 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.40

F1 - Processing parameters

SI	1024
MC2	QF
SF	125.7577985 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	27.00 cm
CX1	23.00 cm
F2PLO	8.913 ppm
F2LO	4457.58 Hz
F2PHI	0.045 ppm
F2HI	22.70 Hz
F1PLO	141.545 ppm
F1LO	17800.34 Hz
F1PHI	4.990 ppm
F1HI	627.58 Hz
F2PPMCM	0.32842 ppm/cm
F2HZCM	164.25478 Hz/cm
F1PPMCM	5.93714 ppm/cm
F1HZCM	746.64122 Hz/cm